Evaluation of ensiled whole-crop legumes for ruminants

Thesis

EVALUATION OF ENSILED WHOLE-CROP LEGUMES FOR RUMINANTS

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A thesis submitted in fulfilment of the requirements of the Open University

for the of degree of Doctor of Philosophy

August 2005

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DATE OF SUBMISSION 31 AUGUST 2005
DATE OF AWARD 14 DECEMBER 2005

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Abstract

Experiments evaluating the production methods and nutritive values of ensiled whole-crop legumes for ruminants were conducted. The initial experiment studied the effect of direct-cutting or wilting and additive type on ensiling profile and nutritive value of whole-crop pea (WCP) and bean silages differing in condensed tannin (CT) content. All treatments produced lactic-type silages with a mean DM, pH, CP and NH_3-N content of 209 g kg^{-1}, 4.1, 158 g kg^{-1} DM and 80 g kg^{-1} TN respectively. Mean WCP had a higher (P<0.050) DM, CP, IVDOMD and estimated ME content compared to beans.

In subsequent experiments degradation characteristics of WCP silages differing in CT content were determined. In addition, the effect of dietary inclusion of WCP on lamb growth rate, nitrogen balance and dairy cow milk production were investigated. WCP had a greater (P<0.001) nitrogen degradation and higher (P<0.050) effective degradability compared to grass silage (GS). Lambs were fed diets containing GS or GS/WCP supplemented with barley, with or without an additional 200 g d^{-1} soya-bean meal (SBM). Feeding WCP silage increased (P<0.050) mean growth rate by 21.9 g d^{-1} but forage DM intake was not affected, equating to a SBM replacement equivalent of 95 g d^{-1}. Including WCP silage in diets did not affect lamb nitrogen balance (mean 11.3 g d^{-1}) or microbial protein synthesis (mean 14.8 g N d^{-1}). WCP silage was added to dairy cow rations which were supplemented with either 1.1 kg d^{-1} of SBM or wheat. Performance was evaluated against a control diet supplemented with SBM. Feeding WCP silage in dairy rations increased (P<0.050) forage DM intake in contrast to the control diet. However, no effect on milk or milk component yield (mean 24.0 kg, 1.02 kg fat and 0.83 kg protein d^{-1} respectively) was observed, equating to a daily SBM replacement of 1.1 kg.

DECLARATION

This thesis has been composed by myself and has not been accepted in any previous application for a degree. The work, of which this thesis is a record, has been completed by myself and all sources of information have been acknowledged by means of references.

Kenton James Hart

Acknowledgments

I would like to thank my director of studies, Dr. Jim Huntington and supervisors Dr. Liam Sinclair and Dr. Robert Wilkinson for all of their help, guidance, extreme patience and helpful criticism throughout all of the experiments and the writing of this thesis.

To Edward Holland, Eoin Horgan, Kay Williams, Lesley Askwith, Mathew Lane, Simon Stone, and Tom Falder who assisted in the experimental work as part of their BSc thesis

To Mrs. Chris Dent, Mr. Clive George and Mrs. Janet Pemberton for their assistance with laboratory analysis. To Mr. David Ferguson and Mr. Giles Vince for their invaluable assistance with the animal studies.

To the CERC team and Mr Rhys Davies for their agronomic skills and advice.

To all of the post graduate students who have helped at some stage with either producing silage, fighting with cows and sheep or discussing ideas, especially Judith Capper, Marie Jackson and Shell Lewis.

To Mrs. Delma Jones and the Institute of Grassland and Environmental Research for the analysis of volatile fatty acids.

To Dr. Bren Hector and the Macaulay Institute for the determination of purine derivatives.

To my family for their continued support and encouragement.

Finally, to The Perry Foundation for their financial backing of the project and to Harper Adams for my studentship and supplying the resources to enable me to complete the work.

I am deeply indebted to all of you, THANKYOU!

Table of contents

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADIN	Acid detergent insoluble nitrogen
CP	Crude protein
CT	Condensed tannin
d	Days
DE	Digestible energy
DM	Dry matter
DUP	Digestible undegradable protein
ERDP	Effective rumen degradable protein
FiM	Feed into milk
FM	Fresh matter
FME	Fermentable metabolisable energy
g	Gram
GE	Gross energy
GM	Genetically modified
h	Hour
HYT	Hydrolysable tannin
kg	Kilogram
L	Level of feeding
LAB	Lactic acid bacteria
LU	Livestock unit
MCP	Microbial crude protein
ME	Metabolisable energy
MJ	Megajoule
MP	Metabolisable protein
N	Nitrogen
NDF	Neutral detergent fibre
NFC	Non fibre carbohydrates
NH3-N	Ammonia nitrogen
NPN	Non protein nitrogen
OM	Organic matter
P	Probability
PN	Protein nitrogen
s.d.	Standard deviation
s.e.d.	Standard error of the difference
t	Tonne
TN	Total nitrogen
TP	True protein
WSC	Water soluble carbohydrates
xg	Relative centrifugal force (x gravity)

1.0 LITERATURE REVIEW

1.1 Introduction

In the UK, the predominant source of protein utilised in ruminant livestock is from grazed and preserved grassland (Entec, 1997). During 1995 this represented approximately 0.72 of total ruminant protein requirements (Wilkins and Jones, 2000). The crude protein (CP) content of grazed grass and grass silage are normally in the range of 150-220 and 100-160 g CP kg^{-1} DM, respectively, depending upon growth stage, season and the timing and amount of nitrogen (N) based fertiliser applied (Wilkins and Jones, 2000).

It has been estimated by Entec (1997) that the shortfall between total protein requirement for all livestock and total home-grown protein production in the UK is approximately 10% per annum. Since the European wide ban on mammalian derived protein products (EC, 1994), following the bovine spongiform encephalopathy epidemic in the UK, the European Community has relied on the use of soya-bean meal (*Glycine max*) and fishmeal to fulfil the protein deficit (Entec, 1997). However, the use of fishmeal and other animal derived protein products in ruminant diets throughout Europe was banned temporarily (1st January 01: EC, 2000) and then permanently (1st July 01: EC, 2001), increasing the reliance on imported soya-bean meal, mainly from Brazil (Merry *et al.*, 2001). After solvent or pressure extraction of soya oil, the residual meal contains 497 g CP kg^{-1} DM (AFRC, 1993). The price and availability of soya-bean meal as an animal feedstuff is regulated by the global demand for soya in human consumption (Merry *et al.*, 2001). Entec (1997) reported that the UK imported 1.5 million tonnes (t) of oilseed cakes/meals (mainly soya) in 1995, of which 19,500 t was utilised in ruminant livestock production systems.

Production of silages, from grass, clover and mixed grass/clover swards, for over-winter feeding of ruminants, within England has reduced by approximately 936,000 t FM per year

from 1992 to 2003 (r^2=0.935, calculated from DEFRA, 2004), whereas the production of silages from other arable crops, such as wheat (*Triticum aestivum*), barley (*Hordeum sativum*) or maize (*Zea mays*), has increased by approximately 187,000 t FM per year (r^2=0.778, calculated from DEFRA, 2004), during the same period. The increase in production and feeding of whole-crop cereal silages, which have a high starch content and generally contain less crude protein than the grass silage that they are replacing, has the resultant effect of increasing the need for protein supplementation (Entec, 1997; Anil *et al.*, 1998).

This study aims to test the following null hypotheses

- Silages cannot be made from whole-crop annual legumes without extensive wilting or without large quantities of silage additives.
- Silages produced from whole-crop legumes cannot replace the protein supplied from soya-bean meal in the diets of ruminants.
- There is no difference in ensiling characteristics, rumen degradability or animal performance between whole-crop leguminous silages varying in condensed tannin content.

1.2 Home-grown protein

1.2.1 Concentrate production

The main home-grown protein concentrate source in the UK is rapeseed meal produced from oilseed rape (Brasica napus: Entec, 1997), which after oil extraction contains on average 400 g CP kg^{-1} DM (AFRC, 1993). The area of oilseed rape grown in England has increased sporadically between 1994 and 2003 with an average increase of 13,300 ha per year (r^2=0.495) with a total area of 451,000 ha in 2003 (calculated from DEFRA, 2004). The Blair House Agreement (BHA), between the EU and the US, has restricted the production of oilseeds (rape, sunflowers and soya) within the EU to a total area of 5.482 million ha since 1992 (Verhoog, 2002). Under the conditions of the BHA, farms within the EU had to commit 10% of their land to set-aside in order to receive crop specific arable area aid. However, since the Agenda 2000 CAP reform the subsidies on oilseeds have declined from over €81 t^{-1} to €63 t^{-1}, the same as that for cereals (Verhoog, 2002). It is unclear whether this reduction in crop specific subsidies will release the EU from the BHA constraints (EC, 2002) and in turn increase oilseed production, or whether the increase in size of EU will encourage the re-negotiation of the BHA at a future World Trade Organisation meeting.

Both soya and oilseed rape have successfully been genetically modified (GM: Anil *et al.*, 1998), and are grown in the Americas, but not in the UK. Soya-bean meal grown in Brazil is considered GM free. Increased production has, however, resulted in increased areas of Amazon deforestation to supply sufficient fertile land (Merry *et al.*, 2001). With public anxiety over the safety and ethics of GM feedstuffs (Anil *et al.*, 1998), traceablity becomes of increasing concern. Therefore, there is increasing pressure on producers and suppliers to supply cheap, traceable, quality goods (Merry *et al.*, 2001).

Globally, the grains from beans (*Vicia faba*) and peas (*Pisum sativum*) are ranked 3rd and 4th respectively, after soybeans and peanuts (*Arachis hypogaea*) in terms of food production from legumes (Vidal-Valverde *et al.*, 2003). However, Wilkins and Jones (2000), reported that beans and peas only represented 0.4% of the CP supply to UK ruminants in 1995. The reluctance of UK farmers to increase production of beans and peas has historically been related to the higher arable area payments for cereal crops and the abundance of cheap N based fertilisers (Entec, 1997; Merry *et al.*, 2001). However, due to the Common Agricultural Policy reform resulting in the decoupling of payments linked to production (EC, 2002), the introduction of nitrate vulnerable zones and increasing consumer demand for organic produce, the use of legumes, which have a symbiotic relationship with the N fixing bacteria *Rhizobia* species (spp.), is likely to increase (Merry *et al.*, 2001). Bergersen (1973) reported that 75 kg N ha^{-1} could be fixed naturally by the symbiotic *Rhizobia* bacteria. Fixed N can also contribute directly to a companion crop or indirectly to a following crop (Wilkins *et al.*, 2001).

1.2.2 Forage legumes as protein crops

Merry *et al.* (2001) stated that the forage legumes red and white clover (*Trifolium pratense* and *T. repens* respectively) and lucerne (*Medicago sativa*) had historically played a major role in UK agriculture, but the increasing demand for cereal production had excluded the use of these legumes in arable rotations. However, their use, along with the forage legumes sainfoin (*Onobrychis vicifolia*), birds-foot trefoil (*Lotus corniculatus*), and forage peas and beans, are now being examined in more detail. The production of determinate varieties of white lupins (*Lupinus albus*) for seed, within the UK has not been widely established due to little on farm experience, and their specificity for acidic soils which are neither very light or heavy. This limits the potential growing area to 100,000 ha in the UK (Entec, 1997). This represents only 0.5% of the total arable land. Forage legumes offer the potential to be grazed, ensiled or used to make hay, but not all of the aforementioned

legumes are suited for all three types of utilisation. Their suitability is summarised in Table 1.1. It is apparent from Table 1.1 that grass is the most flexible forage that can be grown in the UK. However, in order to support high DM yields a large amount of N fertiliser (>200 kg ha^{-1}) is typically applied (Chadwick, 2004).

Table 1.1 Suitability of grass and forage legume crops for ease of production, grazing, ensiling and hay production under UK climatic conditions

Crop	Agronomy	Grazing	Silage	Hay
Grass	✓✓✓	✓✓✓	✓✓✓	✓✓
Grass and white clover	✓✓	✓✓	✓	✓
Red clover	✓✓	✓	✓	×
Lucerne	✓	✓	✓✓	✓
Sainfoin	✓✓	✓✓	×	✓
Birds-foot trefoil	✓✓	✓✓	?	✓
Forage peas	✓✓	×	✓✓	×
Forage beans	✓✓	×	✓✓	×

✓✓✓ Good, ✓✓ Moderate, ✓ Poor, × not suitable, ? Unknown. (adapted from Entec, 1997)

A recent European wide collaborative study examining legume silages for animal production (LEGSIL: Wilkins *et al.*, 2001), evaluated the use of the perennials white clover, red clover, lucerne, lotus (birds-foot trefoil) and galega (*Galega orientalis*), in a three cut silage system over two consecutive years. Halling *et al.* (2001) concluded that all of the legumes studied had a higher ensiling buffering capacity (Section 1.3.2), digestible organic matter content, CP content, estimated metabolisable energy and a lower water soluble carbohydrate content compared to fertilised (200 kg N ha^{-1}) grass at point of harvest. Sheldrick *et al.* (1995) reported that white lupins grown in France for ensilage had a good DM yield ranging between 8 and 9 t DM ha^{-1}, with a low CP content of 150 g kg^{-1} DM, although their production within the UK has shown little potential. The production of silage from whole-crop white lupins has recently been evaluated in the UK by Fraser *et al.* (2005), from which it was concluded that whole-crop lupins had a low DM yield of 5.95 t ha^{-1} and a moderate CP of 219 g kg^{-1} DM representing 64% of the expected CP of 342 g kg^{-1} DM for lupin seed meal (AFRC, 1993).

Forage peas and beans are grain producing, annual crops requiring re-establishment each year and are hence unsuitable for grazing or hay production (Table 1.1). Second only to grass, the legumes forage peas and beans seem to be the most suitable crops to establish and harvest for ensilage within the UK due to climatic suitability, competitive growth habits and short growing season (Table 1.1, Entec, 1997).

1.2.2.1 Agronomy of peas and beans

Peas and beans can either be winter or spring sown; the winter-sown varieties tend to out yield their spring-sown counterparts (NIAB, 2001). The recommended establishment methods for winter and spring sown beans and peas are summarised in Table 1.2. It is recommended that only winter beans should be sown using a broadcast method followed by a shallow ploughing because the seed size is often too large for use in a conventional cereal drill.

Table 1.2 Cultural establishment recommendations of winter and spring sown beans and peas

	Sowing method		Sowing depth†	Row spacing	Target plant population (m^{-2})	% field loss
	B/cast	Drilled				
Winter beans	✓	✓	7.5 or 3 cm	17-35 cm	18	22.5
Spring beans	×	✓	7.5 or 3 cm	17-35 cm	40	7.5
Winter peas	×	✓	3 cm	Max 20 cm	80	20
Spring peas	×	✓	3 cm	Max 20 cm	70	12.5

✓ Suitable, × Not suitable. † Sowing depth after rolling. Adapted from NIAB (2001)

The optimum drilling time for winter beans is mid-October to early November whereas the optimum time for winter peas is the first two weeks of November (NIAB, 2001). The optimum time for drilling spring sown beans and peas is early to mid March (NIAB, 2001). The difference in sowing depth of beans, i.e. 7.5 or 3 cm, is related to the choice of pre-emergence herbicide, with the use of simazine requiring the deeper sowing depth (NIAB, 2001). The typical row spacing of peas and beans is closely related to cereal plant spacing due to the use of a cereal drill (NIAB, 2001). The target population of winter beans is lower than that of spring beans whereas the reverse is true in peas. This is because winter

peas are less winter hardy and are smaller than winter beans (NIAB, 2001). Sheldrick *et al.* (1995) recommend that both peas and beans should be grown in soils with a pH of 6.0, but peas should be sown in lighter, well draining soils whereas beans prefer heavier, moisture retentive soils, although water logging is a problem in both species. It has been recommended by NIAB (2001) that if the soil indexes of phosphate (P_2O_5) and potassium (K_2O) are below 2 prior to the establishment of peas and beans, then the application of fertiliser is recommended (Table 1.3). There is no need for the application of N-based fertiliser to a leguminous crop (Bergersen, 1973).

Table 1.3 Nitrogen (N), phosphate (P_2O_5) and potassium (K_2O) fertiliser requirements of peas and beans in relation to soil index (kg ha^{-1})

Soil N, P, K index		Peas			Beans		
Numeric	Level	N	P_2O_5	K_2O	N	P_2O_5	K_2O
0	Very low	0	50	150	0	75	120
1	Low	0	25	40	0	50	50
2	Medium	0	0	0	0	0	0
>2	High	0	0	0	0	0	0

Adapted from NIAB (2001)

When fertiliser application is necessary it should be broadcast and shallowly ploughed in order to maximise its utilisation by the crop (NIAB, 2001). Potts (1982) examined the yield response of forage peas to the application of 0, 20, 40 and 60 kg N ha^{-1} with either 0, 40 or 80 kg K_2O ha^{-1} and found that there was no difference in DM yield of forage between any of the treatments, although it was assumed that the addition of potash would increase seed yield at a later harvest. Bergersen (1973) reported that the addition of N based fertiliser to an established leguminous crop resulted in a lower N uptake by the plant, due to an inhibitory effect of fertiliser N on the symbiotic Rhizobium bacteria.

Anil *et al.* (1998) stated that peas and beans were both naturally very competitive, generally able to outgrow broadleaf weeds, thus reducing the need for herbicides. It has been recommended by the Pulse Growers and Research Organisation that peas, beans, vetches, linseed and oilseed rape should not be grown on the same site more than one year

in five due to the longevity of soil borne pathogens such as *Fusarium solani*, *Phoma medicaginis* and *Sclerotina sclerotiorum* (NIAB, 2001).

The growth stage of cereal plants is defined by a decimal growth stage developed by Zadoks *et al.* (1974), but this is inappropriate for use in peas and beans. A growth stage key for the development of peas was developed by Knott (1987), and for beans by Knott (1990). Table 1.4 details the growth stages of beans and peas.

Peas varieties have traditionally been classed into three types; vining peas, forage peas and combinable (dried) peas (Brokenshire *et al.*, 1983). However, market peas, i.e. those from which whole pods are harvested for human consumption, are being recognised as a fourth type (Cousin, 1997). Forage varieties of peas are generally tall and leafy, with weak, fleshy stems, that are typically grown for silage either as a monoculture or mixed with a cereal crop (Brokenshire *et al.*, 1983). Dried pea seeds, harvested at growth stage 303 (Knott, 1987), can be included in the rations of both ruminants and monogastrics (Entec, 1997), and the straw can either be baled for feeding or ploughed back into the ground. Field beans are predominantly grown for the harvest of dried seed (Duc, 1997), corresponding to growth stage 410 (Knott, 1990), and can be included into both ruminant and non-ruminant rations. However, many field varieties of beans and combinable varieties of peas lend themselves to being used for silage production. Harvesting peas and beans for forage requires the same machinery as used for grass. However, care must be taken in the choice of mower, since mowers fitted with conditioners may lead to extensive pod loss at harvest (McDonald *et al.*, 1991), reducing nutritive value.

The use of peas in the diets of monogastrics has received little attention due to antinutritional factors and the low biological value of pea protein (McDonald *et al.*, 1998). Work by Hlodversson (1987) demonstrated that the biological value of pea seed meal in porcine rations was similar to that of barley with an average value of 0.624.

Table 1.4 Definitions and codes for the stages of development of beans and peas

Growth stage	Bean	Pea
Germination and emergence		
000	Dry seed	Dry seed
001	Imbibed seed	Imbibed seed
002	Radicle apparent	Radicle apparent
003	Plumule & radicle apparent	Plumule & radicle apparent
004	Emergence	Emergence
005	1st leaf unfolding	---
006	1st leaf unfolded	---
Vegetative stage		
101	1st node	1st node
102	2nd node	2nd node
103	3rd node	3rd node
10x	x node	x node
10n	n last recorded node	n last recorded node
Reproductive stage†		
201	Flower buds visible	Enclosed buds
202	---	Visible buds
203	1st open flowers	1st open flower
204	1st pod set	Pod set
205	Pods fully formed and green	Flat pod
206	---	Pod swell
207	Pod fill, pod green	Pod fill
208	---	Green wrinkled pod
209	Seed rubbery, pods turning black	Yellow wrinkled pod
210	Seed dry and hard, pods black	Dry seed
Pod senescence stage		
301	10% pods dry and black	Lower pods dry and brown
302	20% pods dry and black	Lower and middle pods dry and brown
303	30% pods dry and black	All pods dry and brown
30X	X% pods dry and black	---
309	90% pods dry and black	---
310	All pods dry and black, seed hard	---
Stem senescence stage		
401	10% stem black/brown	---
40X	X% stem black/brown	---
409	90% stem black/brown	---
410	All stem black/brown	---

† On 1st fertile node (beans) or on main stem (peas). Dashed line indicates non-applicable growth stage. Adapted from Knott (1987; 1990).

Peas and beans used for grain production grow well in all areas of the UK, however care should be taken when selecting bean varieties to be grown in Scotland, due to their lateness in ripening and the tendency for rain in late summer, increasing the risk of lodging prior to

harvest (NIAB, 2001). Work by Fraser *et al.* (2001) has shown the potential of spring sown peas and beans to be harvested for ensilage between 10 and 14 weeks post sowing, allowing an early harvest which does not clash with harvesting of cereals.

1.2.2.2 Important genetic traits of peas and beans

The development of semi-leafless peas is controlled by the af (afila) gene (Cousin, 1997), which results in the production of tendrils instead of normal leaves. The genetic combination of the st (stipule) and af gene results in leafless peas (Cousin, 1997). Cousin (1997) reported that the af gene in semi-leafless peas resulted in a better standing ability, a 40% reduction in leaf area and a better distribution of the leaves along the stem, which allowed light to penetrate through the canopy more easily. The entanglement of the tendrils between pea plants resulted in an increased standing ability (Koivisto, 2001).

In early experiments by Mendel (1865) with peas, it was found that flower colour was controlled by a single gene pair, with the dominant gene 'A' coding for red/violet flower colour and the recessive gene 'a' coding for white flowers. Therefore peas containing the gene combinations 'AA' or 'Aa' had coloured flowers and those containing the gene combination 'aa' had white flowers. It has since been identified that the coloured flower gene 'A' is analogous with the presence of condensed tannin (e.g. Ma and Bliss, 1978; Crofts *et al.*, 1980; Carbrera and Martin, 1989) and in a study by Helsper *et al.* (1993) it was concluded that the homozygous recessive pair ('aa') resulted in the absence of condensed tannin and testa-bound trypsin inhibitor in near isogenic varieties of faba beans.

1.2.2.3 Effect of growth stage on chemical composition of peas and beans

Tisserand and Roux (1976) reported that the CP of whole-plant beans increased to a maximum at flowering, corresponding to growth stage 203, and then decreased towards

onset of senescence. Table 1.5 summarises the changes in DM, CP, non-structural carbohydrates and buffering capacity of whole-crop peas and beans in relation to three growth stages. The CP content of both the peas and beans decreased from growth stage 204, with the maximum CP yield being at growth stage 204 and 207 respectively. The DM content of both peas and beans increased as time post sowing increases, with a maximum DM yield being observed at growth stage 205 and 207 respectively. Water soluble carbohydrate concentration was highest at growth stage 204 in both peas and beans. The buffering capacities of peas and beans were highest at growth stage 204 and lowest at growth stage 207.

Table 1.5 Effect of harvest date on the chemical composition and buffering capacity (BC) of spring sown peas and beans at harvest

	Peas			Beans		
	10 weeks	12 weeks	14 weeks	10 weeks	12 weeks	14 weeks
Growth stage[†]	204	205	207	204	205	207
DM g kg^{-1} FM	152	154	206	121	135	153
DM yield kg ha^{-1}	5593	6172	5596	3698	5167	7760
CP g kg^{-1} DM	203	157	159	213	187	180
CP yield kg ha^{-1}	1135	969	890	788	966	1397
WSC g kg^{-1} DM	95	118	84	88	104	97
Starch g kg^{-1} DM	65	75	91	45	73	64
ADF g kg^{-1} DM	304	310	314	287	291	298
BC mequiv. 100 g^{-1}	255	234	193	392	345	341

[†] As described by Knott (1987; 1990). After Fraser *et al* (2001)

It was concluded by Fraser *et al* (2001), that the optimum time for harvesting peas and beans as a whole-crop was at growth stage 205 and 207 respectively.

1.3 Overview of the ensiling process

In order to produce a well preserved, aerobically stable and acidic silage, McDonald *et al.* (1991) stated that the crop to be ensiled should have the following characteristics; firstly it should contain adequate soluble sugars to support bacterial growth, secondly, a relatively low buffering capacity and thirdly, a DM content above 200 g kg^{-1}. Smith *et al.* (1986) suggested that a hexose (glucose/fructose) content of 30-40 g kg^{-1} DM was adequate for the production of silage from a crop with a low buffering capacity. However, this level increases to in excess of 65 g kg^{-1} DM for a highly buffered crop such as legumes. Total water soluble carbohydrate (WSC) content of crops is affected by numerous factors, predominantly growth stage, time of day and weather conditions (McDonald *et al.*, 1991).

Young crops contain a higher proportion of WSC compared to older more fibrous crops, due to the difference in the proportioning of carbohydrate to structural and storage compounds (Waite and Boyd, 1953). The WSC content of grasses increases throughout the day peaking in the afternoon, whereas the WSC content of legumes peaks at midday (McDonald *et al.*, 1991). Mackenzie and Wylam (1957) concluded that the daily duration of sunshine had the largest effect on grass and clover WSC content, due to an increase in hexose biosynthesis from photosynthesis. Smith (1973) concluded that the decrease in legume WSC after midday was due to an increase in the conversion of sucrose to starch. Smith *et al.* (1986) reported that members of both the Leguminoseae (legumes) and Gramineae (grasses) accumulated starch in their seeds. However, due to the stage of growth and the small seed size of temperate grasses and perennial legumes harvested for ensilage, the starch content was negligible. Work by Fraser *et al.* (2001) with the annual, grain producing legumes, peas and beans, demonstrated that the initial starch content of peas and beans ranged from 45 to 91 g kg^{-1} DM (Table 1.5).

Ohshima and McDonald (1978) stated that between 750 and 900 g kg^{-1} total N present in fresh herbage was in the form of protein, with the rest mainly being peptides, free amino acids, ureides, nitrates, chlorophyll, amides and nucleotides. Mangan *et al.* (1991) reported that the most abundant protein (approximately 39% of total protein) in plants was ribulose-1,5-bisphosphate carboxylase (Rubisco), which is only located in the stomata and chloroplasts.

After harvesting, extensive autolytic proteolysis occurs, due to the release of the proteases from the vacuoles due to osmotic pressure (McDonald *et al.*, 1991), resulting in the conversion of true protein nitrogen (PN) to non-protein nitrogen (NPN: Macpherson, 1952a; Brady, 1960; Fairbairn *et al.*, 1992; Davies *et al.*, 1998). McDonald (1973) concluded that the breakdown of protein during ensilage appeared to be non selective. McDonald *et al.* (1991) reported that Rubisco was rapidly broken down during leaf senescence accounting for most of the initial increase in NPN. McKersie (1985) concluded that during ensiling, proteolysis continued to occur until a pH of 4 was achieved which inhibited the activity of the plant proteolytic enzymes.

1.3.1 Stages of ensiling

The ensilage process has been described by Pitt *et al.* (1985) as consisting of 3 phases; aerobic, lag and fermentation prior to the formation of a stable silage. A diagrammatic representation of the qualitative changes over the 3 phases of ensiling is presented in Figure 1.1.

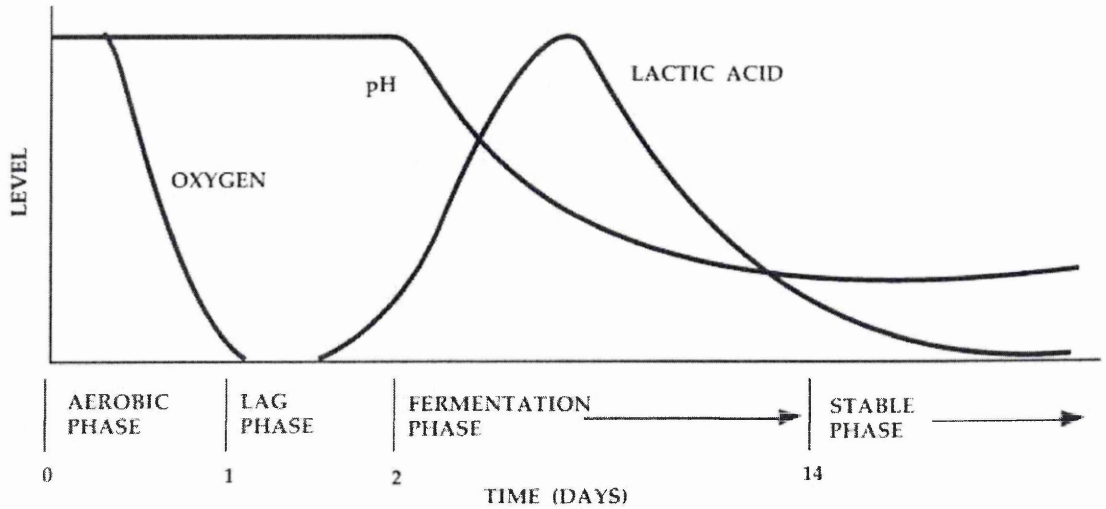


Figure 1.1 The phases of ensilage during the production of a good silage. After Pitt and Shaver (1990)

1.3.1.1 Aerobic phase

The aerobic phase occurs as soon as the plant has been cut, during wilting and during the initial phase in the silo (Woolford, 1984). It is a continuation of normal cellular respiration of soluble carbohydrate via the glycolysis pathway and the tricarboxylic acid cycle and normal plant enzymatic activity (McDonald *et al.*, 1991). During the aerobic phase, energy is released in the form of heat, which is normally dissipated into the environment but, in the case of cut herbage is maintained within the biomass, causing the temperature to rise (McDonald *et al.*, 1991). This in turn stimulates bacterial growth (Woolford and Pahlow, 1998) and increased enzymatic/proteolytic activity (Heron *et al.*, 1989). Gibson (1965) reported that in overheated silage the beneficial lactic acid producing bacteria could be destroyed, but clostridia, which are present in the form of spores on fresh herbage, can survive and subsequently dominate fermentation. The aerobic phase continues until the level of oxygen present in the biomass has depleted to almost zero (Pitt *et al.*, 1985).

1.3.1.2 Lag phase

The lag phase was defined by Pitt *et al.* (1985) as the total time between the start of respiration and the onset of rapid bacterial growth. There are many types of micro-

organisms that are present during ensilage (Figure 1.2), the most essential are the lactic acid bacteria (LAB) which produce lactic acid, and decreases the pH of the ensiling biomass (Woolford, 1984). The numbers of viable LAB bacteria isolated and cultured from fresh herbage are typically less than 10^2 g^{-1} fresh weight (Stirling and Whittenbury, 1963), a level which is insufficient to promote lactic acid fermentation (Woolford and Pahlow, 1998). It was concluded by Roszak and Colwell (1987) that lactic acid bacteria enter a state of dormancy in response to adverse environmental conditions, such as aerobic conditions and lack of substrates for growth, which consequently leads to an under estimation of LAB numbers using culturing techniques, since the LAB measured are viable LAB and not culturable LAB.

The pre-requisite growth medium required for growth of LAB is plant cell juice, which contains the residual soluble carbohydrates (Greenhill, 1964). Watson and Nash (1960) reported that the amount of plant cell juice can be greatly increased by chopping the crop to short lengths using a forage harvester. During the aerobic phase very little plant juice is released, but upon the cessation of aerobic respiration, plasmolysis occurs resulting in the liberation of plant cell juice, a marked subsidence of the ensiling mass and an increased effluent flow (Pitt *et al.*, 1985). Examples of lactic acid producing bacteria are presented in Table 1.6 and their sequence of succession in Figure 1.2.

The changes in LAB genera observed during ensiling are related to the acid tolerance and acidification potential of each genera (Pahlow and Weissbach, 1996). Woolford and Pahlow (1998) reported that enterobacteria (coliforms), which ferment soluble sugars to acetic acid, multiply until about the fifth day from which they decline in numbers. During this period they are progressively replaced by the lactic cocci, which produce lactic acid, which in turn are superseded by the slower growing, more acid producing lactobacilli.

Table 1.6 Lactic acid producing bacteria of importance during ensiling and approximate inhibitory pH values

Genus	Hexose fermentation	Morphology	Species	Inhibitory pH
Enterococcus	Homofermentative	Coccus	*E. faecalis* *E. faecium*	~5.6
Lactococcus	Homofermentative	Coccus	*L. lactis*	~5.0
Streptococcus	Homofermentative	Coccus	*S. bovis*	~5.0
Leuconostoc	Heterofermentative	Coccus	*L. mesenteroides*	~5.0
Lactobacillus	Homofermentative	Rod	*L. acidophilus* *L. casei* *L. coryniformis* *L. curvatus* *L. plantarium* *L. salivarius*	~4.2
	Heterofermentative	Rod	L. brevis *L. buchneri* *L. fermentum* *L. viridescens*	~4.1
Pediococcus	Homofermentative	Coccus	*P. acidilactici* *P. damnosus* *P. pentosaceus*	~4.0

Adapted from McDonald *et al.* (1991) and Woolford and Pahlow (1998)

The end of the lag phase is signalled by the rapid proliferation of LAB and the production of lactic and acetic acid (Pitt *et al.*, 1985). If conditions in the ensiling biomass are not suitable for the rapid proliferation of LAB, i.e. low DM, low WSC content and a high buffering capacity, which are generally conditions associated with legumes (McDonald *et al.*, 1991), then the pH drop is restricted allowing the growth of clostridia (Figure 1.2, Woolford, 1990).

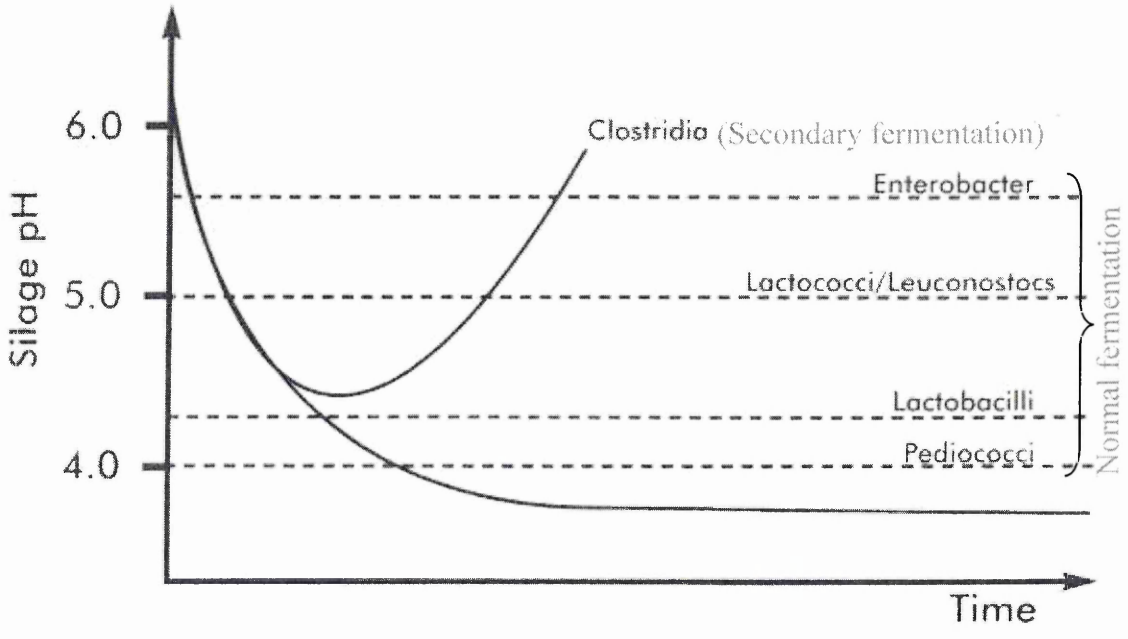


Figure 1.2 Qualitative changes in the silage microbial population during both normal and secondary fermentation. Adapted from Woolford and Pahlow (1998)

1.3.1.3 Effects of clostridia

Clostridia species compete with LAB for the WSC, and initially they can be present in similar numbers (McDonald *et al.*, 1991). The clostridia species ferment glucose and lactate to produce butyrate and acetate, which are weaker acids than lactic acid raising the pH of the ensiling biomass (McDonald, 1973). Butyric acid affects the quality of the resultant silages, with a pungent smell (McDonald *et al.*, 1991) and a decrease in aerobic stability (Woolford, 1990). Ohshima and McDonald (1978) reported that the greatest proteolytic activity observed during fermentation was caused by clostridia. Clostridia catabolise a wide range of amino acids via deamination, decarboxylation and oxidation/reduction (Stickland type) reactions resulting in the production of ammonia, carbon dioxide and butyrate (Gibson, 1965). The formation of ammonia acts as a buffering agent, by acting as a hydrogen sink ($NH_3 + H^+ \leftrightarrow NH_4^+$ (alkaline)), requiring the formation of more acid in order to drop the pH (Ohshima and McDonald, 1978). In addition to amino

acids, clostridia can degrade the purine and pyrimidine bases (Ohshima and McDonald, 1978). A summary of the proteolytic activity of clostridia is presented in Table 1.7.

Table 1.7 Examples of the catabolic reactions of clostridia

Deamination		
3 alanine	→	2 pripoinate + acetate + $3NH_3$ + CO_2
arginine	→	citrulline + NH_3
asparagine	→	aspartic acid + NH_3
aspartic acid	→	fumaric acid + NH_3
glutamic acid	→	mesaconic acid + NH_3
glutamine	→	glutamic acid + NH_3
histidine	→	urocanic acid + NH_3
leucine	→	isobutyrate + NH_3 + CO_2
lysine	→	acetic acid + butyric acid + $2NH_3$
methionine	→	α-ketobutyric acid + methylmercaptan + NH_3
phenylalanine	→	phenyl propinonic acid + NH_3
serine	→	pyruvic acid + NH_3
threonine	→	α-ketobutyric acid + NH_3
trytophan	→	indolepropionic acid + NH_3
tyrosine	→	*p*-hydroxyphenyl propionic acid + NH_3
valine	→	isobutyrate + NH_3 + CO_2
Decarboxylation		
arginine	→IM[1]→	putrescine and CO_2
aspartic acid	→	alanine + CO_2
glutamic acid	→	γ-aminobutryic acid + CO_2
histidine	→	histamine + CO_2
lysine	→	cadaverine + CO_2
phenylalanine	→	β-phenylethylamine + CO_2
serine	→	ethanolamine + CO_2
trytophan	→	typtamine + CO_2
tryrosine	→	tyramine + CO_2
Stickland type[2]		
alnaine + 2 glycine	→	3 acetate + $3NH_3$ + CO_2
Degradation of bases		
adenine guanine	→IM[1]→	glycine + acetic acid + formic acid + NH_3 + CO_2
cytosine	→IM[1]→	β-alanine + NH_3 + CO_2
thymine	→IM[1]→	β-amino isobutyric acid + NH_3 + CO_2
Saccharolytic		
2 lactic acid glucose	→	butyric acid + $2CO_2$ + $2H_2$

[1]IM intermediates, [2] an example. Adapted from Ohshima and McDonald (1978) and Woolford and Pahlow (1998)

McDonald and Edwards (1976) classified a badly preserved, butyrate type silage, as one which had a low dry matter content less than 180 g kg^{-1} FM, an ammonia nitrogen content in excess of 240 g kg^{-1} TN and a butyric acid content of in excess of 35 g kg^{-1} DM.

1.3.1.4 Fermentation phase

The objective of the fermentation phase is to achieve a stable preservation, whilst minimising the loss of nutrients and avoiding adverse chemical changes in the ensiling herbage by the production of lactic and acetic acid by LAB (Jaster, 1995). Kandler and Weiss (1984) subdivided the LAB into three groups depending upon the principal saccharolytic pathways employed by the species;

- Group I, obligate homofermentative; these convert hexoses into lactic acid via the Embden-Meyerhof pathway (Figure 1.3a), and they are unable to utilise pentoses or gluconate as a substrate. *Lactococcus lactis* and *Streptococcus bovis* are important group I LAB involved in ensilage.

- Group II, facultative heterofermentative; these usually ferment hexoses homofermentatively into lactic acid (Figure 1.3a). However, under special conditions, heterofermentative metabolism occurs, forming lactic acid, carbon dioxide and ethanol (Figure 1.3b). Production of acetic acid occurs when NAD^+ can be regenerated without the formation of ethanol, for example through the reduction of fructose. Pentoses are fermented into lactic and acetic acid via a phospoketolase. *Lactobacillus plantarum* and *L. casei* are important in ensilage.

- Group III, obligate heterofermentative (Figure 1.3b); hexoses are fermented to lactic acid, carbon dioxide and ethanol (or acetic acid in the presence of an alternative electron acceptor). Pentoses are fermented to lactic and acetic acids. *Lactobacillus buchneri*, *L. brevis* and *Leuconostoc mesenteroides* are important in ensilage.

The fermentation of hexoses via a homofermentative pathway results in a more efficient yield of lactic acid, i.e. 1 mole of hexose is fermented to 2 moles of lactic acid (Figure 1.3a), whereas heterofermentative fermentation of 1 mole of glucose results in 1 mole of lactic acid and 1 mole of ethanol, and heterofermentative fermentation of 3 moles of fructose results in the formation of 1 mole of lactic acid, 1 mole of acetic acid and 1 mole of mannitol (Figure 1.3b, McDonald, 1973). The preservation effect of lactic acid is dependant upon the DM of the ensiling herbage and the pH, whereas the preservative effect of acetic acid is mainly due to its antimicrobial properties (Holzer *et al.*, 2003).

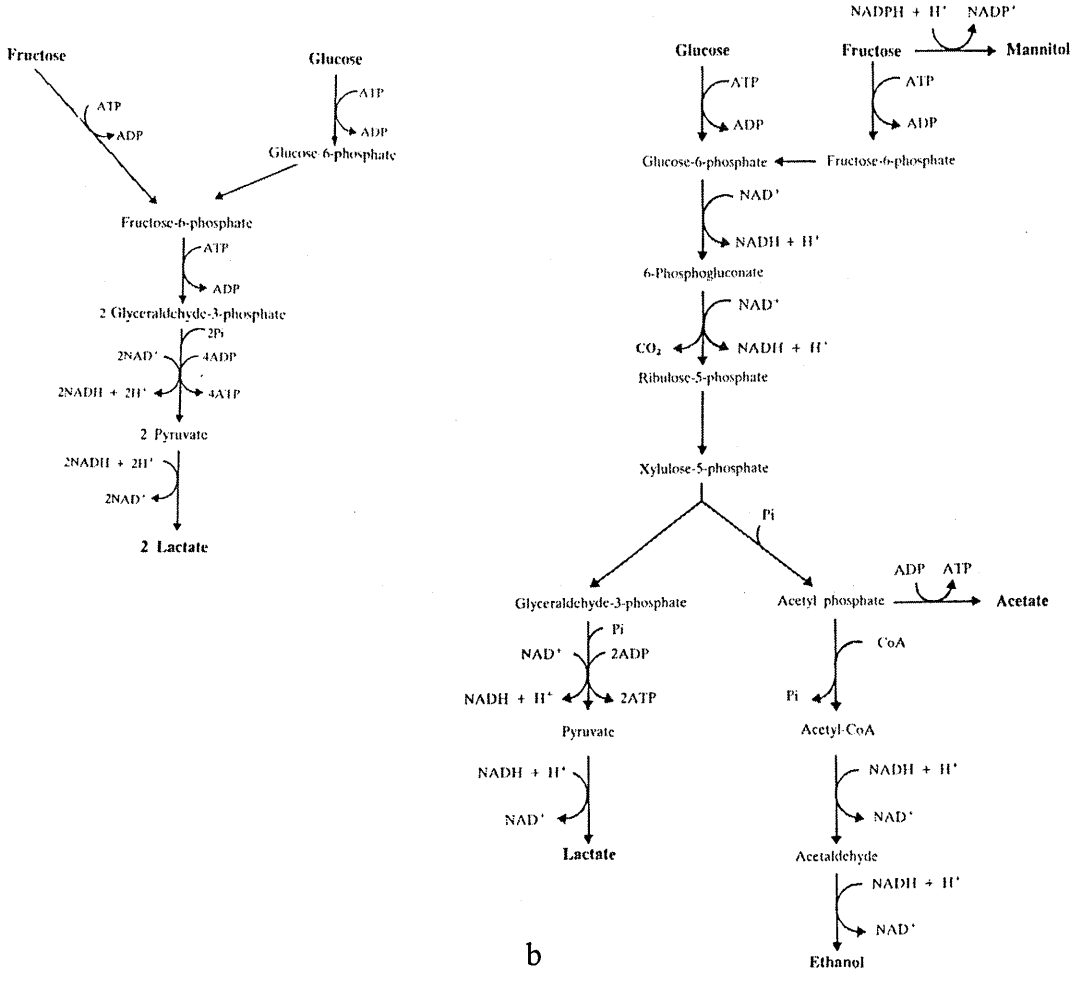


Figure 1.3 a) Homofermentation and b) heterofermentation of hexose sugars by Lactobacillus bacteria during ensiling. After McDonald *et al.* (1991)

Woolford (2000) stated that under natural conditions the heterofermentative LAB dominate over the homofermentative LAB, resulting in an increased acetate to lactate ratio. Lactic

acid is a stronger acid than acetic acid with acid dissociation constants (pk_a) of 3.70 and 4.64 respectively (Woolford, 2000). The LAB have very little proteolytic activity, only possessing the ability to ferment arginine and serine to ornithine and acetoin respectively (Ohshima and McDonald, 1978).

Towards the end of the fermentation phase, regeneration of ATP is limited (Oude Elferink *et al.*, 2001). A proposed pathway for ATP generation from the conversion of lactic acid to acetic acid and 1,2-propnediol by *Lactobacillus buchneri* was proposed by Oude Elferink *et al.* (2001), and is presented in Figure 1.4. In a study by Danner *et al.* (2003) it was shown that using the group III heterofermentative LAB, *Lactobacillus buchneri* and *L. brevis*, as a silage inoculant resulted in an increased aerobic stability of the resultant silages. This effect was attributed solely to the higher acetic acid content of these silages compared to silages treated with homofermentative LAB (Danner *et al.*, 2003). The fermentation phase ends when the pH of the herbage inhibits further growth of the acid tolerant LAB species at approximately pH 4.0 (Table 1.6, Figure 1.2, Pitt *et al.*, 1985).

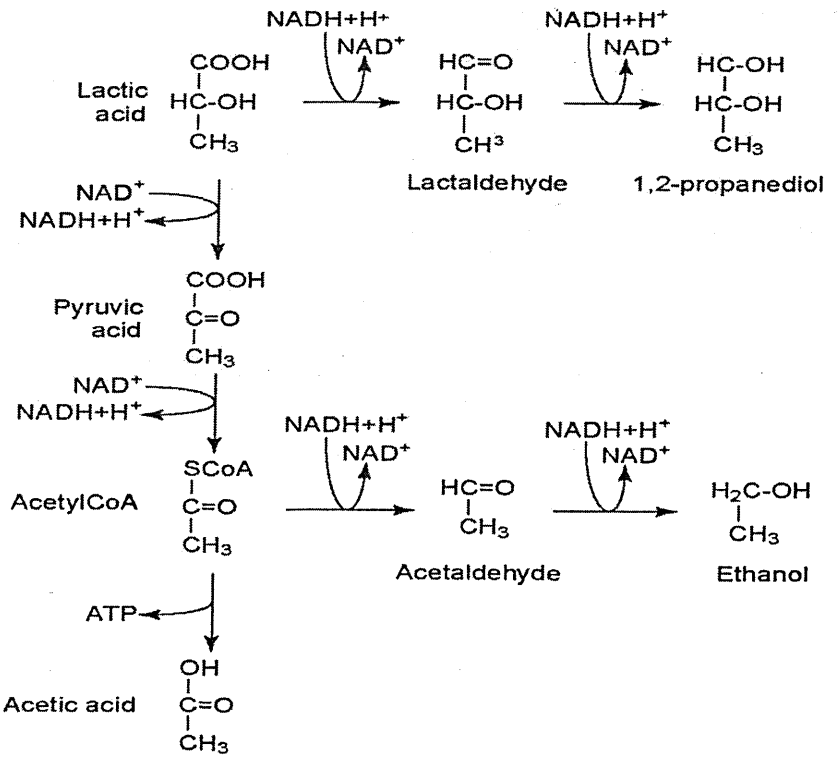


Figure 1.4 Anaerobic conversion of lactic acid to 1,2-propaediol and acetic acid by Lactobacillus buchneri. After Oude Elferink *et al.* (2001)

1.3.2 Buffering capacity

The buffering capacity of plants is their ability to resist change in pH, thus a plant with a high buffering capacity requires the formation of more H^+ ions compared to one with a low buffering capacity (McDonald *et al.*, 1991). It has been recognised for many years that legumes are more highly buffered than grasses (e.g. Watson and Nash, 1960; McDonald and Henderson, 1962; Playne and McDonald, 1966; Halling *et al.*, 2001), an effect that has traditionally been incorrectly associated with the higher protein content of legumes compared to grasses. McDonald and Henderson (1962) compared legumes with similar protein containing grasses and concluded that protein content only accounted for between 10 and 20% of the total buffering capacity.

Playne and McDonald (1966) attributed the buffering capacity of herbage to the concentration of anions (negatively charged compounds) from salts of organic acids, orthophosphates, nitrates and chlorides. Between the range of pH 4.0 to pH 6.0, only the organic acid anions and orthophosphates would act as buffers, but the effect of orthophosphates would be small (Playne and McDonald, 1966). In a comparison between fresh italian ryegrass and fresh red clover, Playne and McDonald (1966) demonstrated that red clover contained 2.5 fold more organic acids compared to grass. Furthermore, it has been demonstrated that wilting of crops prior to ensilage lowers the buffering capacity due to a reduction in organic acid content of the herbage (Playne and McDonald, 1966).

The main organic acids present in fresh leguminous herbage are malic, citric, quinic, malonic and glyceric (Playne and McDonald, 1966), which are readily dissimilated by both homo- and hetero- fermentative lactic acid bacteria during the ensiling process (Whittenbury *et al.*, 1967). The breakdown of citrate and malate by bacteria during ensilage results in the formation of either neutral compounds (e.g. acetoin, 2,3-butandiol and ethanol), salts of short chain organic acids (e.g. lactates and acetates), alkaline released cations (e.g. Na^+, Ca^{++} or Mg^{++}) and carbon dioxide (CO_2: Whittenbury *et al.*, 1967).

The consequence of an increased buffering capacity is that more glucose and fructose need to be fermented, in order to produce larger quantities of lactic and acetic acid to overcome the neutralizing effect of the cations prior to achieving a drop in pH (Playne and McDonald, 1966). Since fresh legumes typically contain less soluble carbohydrates than grasses the achievement of a stable pH during ensilage is more difficult (McDonald *et al.*, 1991).

1.3.3 Effect of wilting

Wilting under favourable climatic conditions, i.e. dry, warm and windy, is the starting point of haymaking (Sullivan, 1973), which is the traditional method of forage preservation for over winter feeding of livestock (Woolford, 2000). Partial wilting of a crop, prior to ensilage, does not only increase the DM but also can also reduce the extent of proteolysis from plant proteases (Brady, 1960) and reduce clostridial activity (Wieringa, 1958). However, wilting in unfavourable conditions can be detrimental through increasing numbers of undesirable aerobic bacteria (i.e. Listeria and Clostridia species, Woolford, 1984), restricting the extent of fermentation (Jackson and Forbes, 1970) and increasing proteolysis (Macpherson, 1952b).

Macpherson (1952b) concluded that plant protease activity was inhibited at a DM in excess of 400 g kg^{-1}, but weather conditions within the UK are unlikely to consistently facilitate this. During a typical field wilt of a grass/white clover sward, it is expected that between 20 and 50% of the PN is hydrolysed to NPN (McDonald *et al.*, 1991). The conditions of the wilt are also important. Work by Macpherson (1952a), Brady (1960) and Carpintero *et al.* (1979) concluded that if grass/clover was wilted in moist conditions proteolysis was more extensive than wilting in less humid conditions. Additionally, if the wilt was rapid there was little change in PN content between the fresh herbage and the resultant silages. It was concluded by Brady (1960) that the final distribution of N within the crop pre-ensiling

was directly related to the rate at which moisture is lost. Hristov and Sandev (1998) reported decreased levels of NPN, NH_3-N and free amino acid N in wilted lucerne silage compared to the unwilted control. McDonald *et al.* (1965) reported that DM losses from wilted red clover silage were 66% less than those recorded in unwilted red clover silage. McDonald *et al.* (1962) concluded that forages with a low initial DM of 170 g kg^{-1} could be successfully ensiled so long as there was sufficient WSC to sustain fermentation. However, losses of DM, N and fibre were greater in the silages produced from wet herbage.

1.3.4 Silage additives

The primary use of silage additives is to aid in the rapid acidification of the ensiling biomass in order to restrict clostridial growth and produce a good quality, aerobically stable silage (Henderson, 1993). The secondary use of additives is to prevent the breakdown of PN to NPN (McDonald *et al.*, 1991). Silage additives can be classed in many ways depending upon their mode of action; a typical classification, with examples, is presented in Table 1.8.

Additives are used to improve the fermentation of crops that otherwise are not ideal for ensiling, such as those with a low DM, low water soluble carbohydrate content and high buffering capacity (McDonald *et al.*, 1991).

Table 1.8 Classification of silage additives

Class	Form	Intended mode of action	Examples
Direct acidifiers	Inorganic acids	Reduction in pH of silage at outset, including qualitative changes in the microflora	Sulphuric and hydrochloric acids
	Organic acids		Formic and acetic acid
Fermentation inhibitors	Direct acting sterilants	Inhibition of microflora	Formaldehyde
Fermentation stimulants	Substrates	Encourage fermentation by supplying additional fermentation substrate	Molasses, sucrose
	Enzymes	Conversion of non usable polysaccharides to simple fermentable sugars	Celluloytic, amylolytic, xylanases
	Microbial cultures	Establishment of a dominant desirable microflora	Homofermentative and heterofermentative lactic acid bacteria
			Propionic acid bacteria
Specific antimicrobials	Antibiotics	Direct reduction of growth of spoilage micro-organisms	Bacitracin, streptomycin, bronopol
	Other antimicrobial products		Sodium chloride, sodium nitrate, condensed tannins

Adapted from Woolford (1984), McDonald *et al.* (1991) and Salawu *et al.* (1999)

1.3.4.1 Direct acidifiers

Virtanen (1933) developed a method to preserve herbage by direct acidification, which involved the application of either concentrated hydrochloric or concentrated sulphuric acid to herbage in order to immediately drop the pH down to less than pH 4.0. The production of inorganic/mineral acid silages was widely used by the Scandinavians but not by the British, due to problems associated with handling, application and storage of the corrosive chemicals (McDonald, 1973). More recently, the short chain organic acids, formic and acetic have replaced the mineral acids due to environmental concerns and operator safety (McDonald *et al.*, 1991). It has been reported by McDonald *et al.* (1991) that yeasts, which can grow on exposure to oxygen causing aerobic deterioration (Woolford, 1990), are highly tolerant to formic acid and the effect of applying formic acid to ensiling grass results in increased yeast proliferation. Work by Vagnoni *et al.* (1997), with lucerne, has shown that application of sufficient quantities of either formic acid (215 mequiv. kg^{-1} forage) or sulphuric acid (147 mequiv. kg^{-1} forage), in order to achieve an immediate drop

in pH from pH 6.4 to pH 4.0, only slowed enzymatic proteolytic activity in comparison to silage treated with the protein-precipitating agent trichloroacetic acid. However, the level of NPN was reduced in all acid treatments in comparison to the untreated control silage. Work by Polan *et al.* (1998), with lucerne, also resulted in a decreased NPN content due to formic acid compared to the untreated control. In addition, the application of formic acid to lucerne decreased the proportion of rumen soluble protein and increased the proportion of digestible undegradable protein. In a study by Salawu *et al.* (2001b) with pea/wheat bi-crops, the application of formic acid did not result in any significant difference in soluble N or NH_3-N content (g kg^{-1} TN) in comparison to the untreated control, although treatment with formic acid lowered the concentration of both lactic and acetic acids, and increased the amount of rumen bypass protein. Pahlow *et al.* (2001) reported that the legumes studied in the LEGSIL project required a high application rate of 6 l formic acid per tonne fresh weight in order to achieve good preservation characteristics at low dry matter concentrations (<200 g kg^{-1}). Direct acidifiers can also be classed as fermentation inhibitors due to the acid load inhibiting the growth of the microflora (Henderson, 1993).

1.3.4.2 Fermentation inhibitors

The use of formaldehyde as a silage additive is of important interest due to its bacterostatic properties and its ability to bind to plant proteins forming a methylol compound which renders them unavailable to proteolysis (Figure 1.5 Henderson, 1993). The stability of the resulting methyol compound is reduced under the acid conditions of the abomasum, therefore increasing the amount of digestible rumen undegradable protein (McDonald *et al.*, 1991).

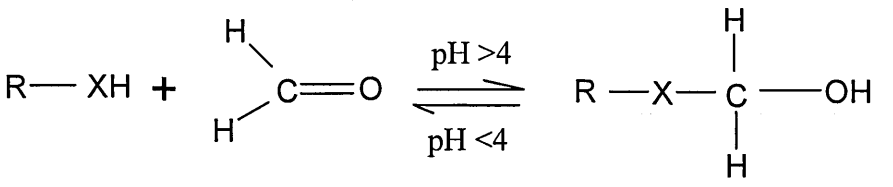


Figure 1.5 Chemical interaction between formaldehyde and protein, where R is the protein and X is the terminal end.

There are a limited number of studies examining the effect of formaldehyde on crops used for ensilage (see review by McDonald *et al.* (1991)). It was concluded by McDonald *et al.* (1991) that an appropriate, but undesirable, level of formaldehyde application to an unwilted legume was in the region of 7 to 9 l per tonne fresh weight, which would result in an approximate pH of 5.5, and in turn would encourage clostridial type fermentation. Formaldehyde must only be used in moderation, as if it remains unbound in the silage it can inhibit the activity of the rumen micro-organisms and may be transferred into milk (McDonald *et al.*, 1991). It has been reported by Hinks *et al.* (1980) that mixing formaldehyde with formic acid increases the protein preservation effect of the additive and increases the levels of residual water soluble carbohydrate in the silage. The application of a formic acid-formaldehyde additive has also been shown to inhibit yeast growth (Hinks *et al.*, 1980). It was reported by Henderson (1993) that although acid-formaldehyde mixes are very effective in preserving forage as silage, their use has been banned in some countries due to the human health risks associated with inhaling formaldehyde vapour (Bolt, 1987).

1.3.4.3 Fermentation stimulants

The practice of adding an additional source of fermentable carbohydrate for subsequent use by LAB has been recognised for many years (Watson and Nash, 1960). Typical carbohydrate sources added to silage are sugar, molasses, whey, citrus pulp and potatoes (Henderson, 1993). Watson and Nash (1960) recommended that glucose should be added to silage in preference to sucrose, since sucrose contains fructose, which when fermented

by heterofermentative LAB forms the neutral product mannitol, although both glucose and sucrose have a high cost. The use of molasses, a bi-product from the sugar industry, is the preferred alternative (McDonald *et al.*, 1991). Work by McDonald *et al.* (1965) concluded that the addition of molasses to red clover, which had a DM content of 142 g kg^{-1} FM, increased the lactic acid content, resulting in a lower pH compared to the unwilted control and decreased the concentration of ammonia nitrogen. However, the addition of molasses to red clover was wasteful because 26% of the water soluble carbohydrate was lost in the effluent compared to only 11% in the untreated control silage, an effect attributable to the low DM of the fresh clover (McDonald *et al.*, 1965). If molasses is used to aid fermentation of ensiling legumes then it has been recommended, in a review by Henderson (1993), that it should be applied at a rate of 40-50 kg per tonne fresh weight to achieve maximum benefit in the resultant silage, i.e. low pH, high residual sugar content and a reduction in proteolysis.

The use of cell wall degrading enzymes, such as hemi-cellulase and cellulase, has two practical effects; firstly, to increase the water soluble carbohydrate content as a substrate for LAB and secondly, to increase the whole-tract digestibility of organic matter of the silage (McDonald *et al.*, 1991). There are very few reported studies evaluating the effect of just enzyme treatment on ensiling, as enzymes are normally mixed with cultures of LAB (McDonald *et al.*, 1991). It has been reported by Henderson (1993) that the optimum pH of cell wall degrading enzymes is between pH 4-5, which makes it unlikely that sufficient sugar will be liberated during the early part of ensiling for the enzymes to be effective. Work by Zhu *et al.* (1999), with lucerne treated with an enzyme mixture of avicelase, carboxymethycellulase and xylanase, resulted in an increased lactic acid content and lower pH compared to the untreated control, in addition to a lower ammonia nitrogen content and a lower acid and neutral detergent fibre content. In a study by Weinburg *et al.* (1995) it was concluded that the application of a mixture of cellulase, hemi-cellulase and LAB to

ensiling peas resulted in a lower pH, higher lactic acid content, a higher residual water soluble carbohydrate concentration and a lower neutral detergent fibre concentration compared to the untreated control. However, the aerobic stability of the treated silages decreased, with a significantly higher yeast count on the treated silages compared to the control.

Watson and Nash (1960) reported that the use of LAB dates back to 1909, when LAB cultures were grown in fresh sugar-beet juice and were then used to inoculate sugar-beet pulp. The resulting product had an increased aerobic stability compared to the non-inoculated pulp. Ideally, a LAB culture should fulfil the following criteria described by Woolford and Sawczye (1984), Seale (1986) and McDonald *et al.* (1991);

1. Rapid growth and successful competition with natural microflora
2. Homofermentation of hexoses and rapid lactic acid production
3. Acid tolerance to pH 4.0
4. Fermentation of a wide range of sugars
5. No production of unfermentable dextran from sucrose
6. No production of mannitol from fructose
7. No degradation of organic acids
8. Growth or survival at temperatures up to 50°C
9. Rapid growth on a low moisture substrate, such as wilted grass
10. Must be able to be formulated in a granular/powder form that remains stable during storage

There are very few strains of LAB that fulfil all of the aforementioned criteria. Woolford and Sawczye (1984) studied 21 strains of LAB and found that none of them fulfilled all of the criteria. However, they did find three strains that fulfilled most of the criteria; *Streptococcus durans*, *Lactobacillus acidophilus* and *Lactobacillus plantarum*. Seale

(1986) reported that most commercial inoculants contain *L. plantarum* in a mixture with other *Lactobacillus* species and either *Enterococcus faecalis* or *Pediococcus* spp. which initiate fermentation and start the decline in pH in order to facilitate fermentation by the *Lactobacillus* spp. Work by Fraser *et al.* (2001) concluded that the application of *L. plantarum* to whole-crop peas and beans ensiled in big bales significantly reduced pH, ammonia nitrogen concentration and acetic acid concentration, and significantly increased lactic acid concentration and residual water soluble carbohydrates compared to the non-treated control.

More recently, Weinburg and Muck (1996) suggested that the inclusion of heterofermentative LAB such as *L. buchneri* may be of benefit by increasing aerobic stability for the reasons discussed earlier (Section 1.3.1.4). Work by Ranjit and Kung (2000), and Kung and Ranjit (2001), examined the use of inoculant based additives containing *L. buchneri* on maize and whole-crop barley silage respectively and concluded that the silages treated with *L. buchneri* had an improved aerobic stability, a lower pH and a reduced number of yeast spores compared to the untreated silages. In a study by Salawu *et al.* (2001b) the application of *L. plantarum* or *L. buchneri* was examined in pea/wheat bi-crops, and it was concluded that there was little difference in chemical composition of the resultant silages except for a lower pH and increased lactic acid concentration observed in those silages treated with *L. plantarum* and an increased acetic acid concentration observed in those treated with *L. buchneri.* In addition, there was no difference between silages treated with *L. plantarum* or *L. buchneri* on resultant aerobic stability. However, both bacterial treatments were less aerobically stable than the untreated control.

It has been reported by McDonald *et al.* (1991) that the addition of propionic acid to silages reduces the extent of deterioration upon exposure to air, an effect that is caused by the antimicrobial effect of propionic acid (Woolford, 1975). Woolford (1975) reported that the antimicrobial effect of propionic acid increased with decreasing pH. The use of

propionic acid bacteria as a silage additive has, however, received little attention. Higginbotham *et al.* (1998) studied the effect of the addition of *Propionibacterium acidipropioici* (propionic acid bacteria), with or without LAB, on the fermentation characteristics and aerobic stability of 230 g kg^{-1} DM maize silage, and concluded that there was no significant difference between pH, lactic acid content, ammonia nitrogen concentration, water soluble carbohydrate concentration, volatile fatty acid profile or aerobic stability of the silages produced. The absence of any significant difference was attributed, by Higginbotham *et al.* (1998), to the low acid tolerance of *P. acidipropioci* in relation to the *Lactobacillus* spp.

1.3.4.4 Specific antimicrobials

The use of antibiotics as silage additives is now considered inappropriate due to the increasing problems with antibiotic resistant bacteria (McDonald *et al.*, 1991). It was reported by Scalbert (1991) that condensed tannins (CT) have antimicrobial activity. In addition, CT have a protein preservation effect that is discussed later (Section 1.4).

Albrecht and Muck (1991) evaluated the effect of intra-cellular tannin in forage legumes on the extent of proteolysis during ensiling and concluded that tannins play a role in limiting proteolysis in some legumes, but other factors were also involved. Salawu *et al.* (1999) studied the use of quebracho tannins, with or without formic acid, compared to formaldehyde, with or without formic acid, as grass silage additives and concluded that the application of an external source of tannin reduced the soluble N and NH_3 content. However, the effect of tannin was not as great as formaldehyde in protecting silage proteins or as effective as formic acid in improving silage quality, but there was a cumulative effect of tannin and formic acid on increasing protein preservation (Salawu *et al.*, 1997). In a further study by Salawu *et al.* (2001b), no difference was observed in the

nutritive value of pea-wheat bi-crop silages treated with no additive, microbial inoculants, formic acid or quebracho tannin.

1.3.5 Ensiling of legumes

Production of whole-crop leguminous silages within the UK has generally been restricted to mixtures of grass and clover, principally due to the reluctance of farmers to ensile monocultures of legumes because of the low DM, low WSC content and high buffering capacity of the crops (McDonald *et al.*, 1991; Entec, 1997).

1.3.5.1 Monocultures

Pahlow *et al.* (2001) have shown that the perennial forage legumes, white clover, red clover, lucerne, lotus and galega, are ensilable as monocultures, but it was recommended that they were harvested at flowering rather than at budding and allowed to wilt to at least 300 g DM kg^{-1} FM with the use of an additive being highly recommended.

Crop production in the UK is dominated by the cereals wheat and barley, which are annual crops and generally require large amounts of inorganic fertiliser in order to achieve high yields (Merry *et al.*, 2001). The establishment of spring sown perennial legumes as a break crop on land suitable for cereal growth may be economically disadvantageous due to the high establishment costs (Chadwick, 2004), whereas the establishment of annual legumes such as white lupins, beans or peas could be economically advantageous due to the production of either a single cut silage or a single grain yield from a quick growing crop (Mustafa and Seguin, 2003). In the UK, perennial forage legumes are more suited for establishment in land designated as temporary grassland (3-5 year ley).

Published values for the production of whole-crop bean and pea silages since 1970 are summarised in Table 1.9 and 1.10 respectively. On average, peas had a higher DM of 296 g kg^{-1} FM (s.d.=79.0) compared to 241 g kg^{-1} FM (s.d.=62.3) for the beans, and a higher

pH, with a mean value of pH 4.2 (s.d.=0.4) and pH 3.9 (s.d.=0.2) respectively. The beans had a higher mean CP content with a value of 204 g kg^{-1} DM (s.d.=14.5) compared to 187 g kg^{-1} DM (s.d.=23.4) for peas, representing proportionally 0.64 and 0.74 of the expected seed CP content respectively (AFRC, 1993). When badly preserved, clostridial type silages are ignored, i.e. NH_3-N >240 g kg^{-1} TN (Section 1.3.1.3), the NH_3-N content of the peas and beans were similar with means of 69 g kg^{-1} TN (s.d.=27.7) and 73 g kg^{-1} TN (s.d.=30.8) respectively. Both pea and bean silages had high NDF contents of 419 g kg^{-1} DM (s.d.=50.9) and 434 g kg^{-1} DM (s.d.=23.4) respectively. On average, whole-crop pea and bean silages exhibited a better nutritive value compared to a good quality grass silage (70D, AFRC, 1993).

Table 1.9 Harvest year , growth stage (GS) and chemical composition of whole-crop bean silages produced since 1970

Crop	Year	Variety	Wilt	Additive	GS	DM	pH	CP	NH_3-N	NDF	ADF	Reference
Vicia faba	1971	Ascott			203†	198		219	141			Tisserand and Roux (1976)
	1971				204†	241		200	102			
	1973				203†	190		204	460			
	1973			FA	203†	195		207	380			
	1973	Diana			209†	330		201	28			Ingalls *et al.* (1979)
	1973		24 h		209†	370		203	28			
	1974	Ackerperle			209†	329		206		444	313	Thorlacius and Beacom (1981)
	1975				209†	342	4.3	199		487	286	
	1976				209†	295	4.2	190		466	322	
	1990	Multiple[a]	24 h+		207	271		155		437	329	Kristensen (1992)
	2000	Maya	24 h‡		204	165	4.2	225	99	401	387	Fraser *et al.* (2001)
	2000		24 h‡	LAB	204	169	4.0	222	67	405	387	
	2000		24 h‡		205	216	3.9	205	79	446	401	
	2000		24 h‡	LAB	205	218	3.7	198	54	438	392	
	2000		24 h‡		207	197	3.8	205	82	436	376	
	2000		24 h‡	LAB	207	199	3.6	202	47	417	358	
	2000		48 h		207	188	3.8	204	77	429	383	
	2000		48 h	LAB	207	197	3.7	202	75	407	376	
	2002	Fatima	12 h+		207	261		222		428	313	Mustafa and Seguin (2003)
					206[x]	**241**	**3.9**	**204**	**204**	**434**	**356**	**Mean**

†growth stage estimated using written description, ‡forced wilt, [a]average of multiple varieties, FA formic acid, LAB lactic acid bacteria, [x] growth stage does not exist

Table 1.10 Harvest year, growth stage (GS) and chemical composition of whole-crop pea silages produced since 1970

Crop	Year	Variety	Wilt	Additive	GS	DM	pH	CP	NH_3-N	NDF	ADF	Reference
Pisum sativum	1975	Trapper			207†	292	4.4	169		482	282	Thorlacius and Beacom (1981)
	1976				207†	361	4.4	204		393	321	
	1990	Multiple[a]	24 h+		207†	310		149		415	261	Kristensen (1992)
	1994			LAB	205†	295	4.3	188		355	317	Weinburg *et al.* (1995)
	1994		24 h+			454		135		445	257	Corbett *et al.* (1995)
	1997	Grande			207†	270		170		371	307	Mustafa et *al.*(2000)
	2000	Magnus	24 h‡		204	268	4.7	228	111	484	401	Fraser *et al.* (2001)
	2000		24 h‡	LAB	204	276	3.8	227	35	455	382	
	2000		24 h‡		205	297	4.0	200	61	417	364	
	2000		24 h‡	LAB	205	293	3.7	199	49	424	361	
	2000		24 h‡		207	280	4.1	204	62	436	365	
	2000		24 h‡	LAB	207	288	3.6	199	42	406	328	
	2000		48 h		207	264	3.9	184	73	428	364	
	2000		48 h	LAB	207	268	3.7	186	50	412	347	
	2000	Capella	24 h		203†	382	4.7		53			Rondahl and Martinsson (2002)
	2000		24 h		205†	319	4.2		77			
	2000		24 h		207†	378	4.8		55			
	2000			FA	203†	155	4.4		76			
	2000			FA	205†	153	4.8		121			
	2000			FA	207†	196	3.9		123			
	2001	Magnus	48 h		206	528	4.5	158	41	535	314	Salawu *et al.* (2002b)
	2001	Lenca	24 h+		207†	265	4.0	179		427	318	Mustafa *et al.* (2002)
	2001	Carneval	24 h+		207†	280	4.0	205		317	252	
	2001	Delta	24 h+		207†	274	4.0	190		333	253	
	2002	Carneval	12 h+		207	250		178		416	312	Mustafa and Seguin (2003)
					206	**296**	**4.2**	**187**	**69**	**418**	**321**	**Mean**

†growth stage estimated using written description, ‡forced wilt, [a]average of multiple varieties, FA formic acid, LAB lactic acid bacteria

1.3.5.2 Bi-crops

Due to the perceived difficulties in producing silages from monocultures of annual legumes, there has been increased interest in the production of bi-crops. Bi-crops are produced when a cereal is undersown with a legume (Anil *et al.*, 1998). Recent experiments examining ensiling of pea-wheat bi-crops preserved as silage include Adesogan *et al.* (2002) and Salawu *et al.* (2001a; 2001b; 2002a; 2002b) and a recent example of a bean-wheat intercrop, non-ensiled forage was reported by Ghanbari-Bonjar and Lee (2003). The advantages claimed for the production of bi-crop silage is a decrease in crop lodging, a higher DM yield, less effluent and a higher WSC content compared to the legume alone (Salawu *et al.*, 2002b). The chemical composition and *in vitro* DOMD of the pea-wheat bi-crop silages produced by Adesogan *et al.* (2002) are summarised in Table 1.11. The respective stage of maturity of the peas and wheat at cut 1 were growth stage 207 (Knott, 1987) and early milk stage (Zadoks *et al.*, 1974), and growth stage 209 and early dough stage at cut 2.

Table 1.11 Effect of pea to wheat ratio and harvest maturity on the chemical composition and in vitro DOMD of pea-wheat bi-crop silages (all g kg^{-1} DM unless otherwise stated)

	Pea: wheat ratio (PW)					Significance [a]		
	3:1		1:3					
	Cut 1	Cut 2	Cut 1	Cut 2	s.e.d.	PW	Cut (C)	PW x C
DM g kg^{-1} FM	284	298	308	386	12.3	**	**	*
pH	4.4	4.3	4.0	4.2	0.20	NS	NS	NS
CP	171	197	159	152	3.4	***	*	**
NH_3-N g kg^{-1} TN	102	88	105	89	9.0	NS	*	NS
WSC	11.1	19.4	11.5	30.7	0.40	***	***	***
Starch	86.5	124	106	155	11.9	*	**	NS
NDF	532	558	566	570	4.7	**	*	*
Lactic acid	41.6	50.6	27.0	20.2	0.72	***	NS	***
Acetic acid	49.4	35.0	55.0	31.4	0.30	**	***	***
Propionic acid	7.66	4.97	11.9	7.01	0.600	**	***	NS
Butyric acid	1.67	0.76	0.91	0.55	0.270	NS	*	NS
DOMD	544	537	511	559	11.4	NS	NS	*

[a] NS $P>0.05$, * $P<0.050$, ** $P<0.010$, *** $P<0.001$. After Adesogan *et al.* (2002)

The DM content of the bi-crops increased with increasing wheat level and with increasing stage of maturity. The pH of the silages was similar across all treatments with a mean of pH 4.2. The CP content was higher with the 3:1 pea:wheat ratio silages but differed with

stage of maturity. Ammonia N content was lowest at cut 2 and the total non-structural carbohydrate content increased with both maturity and increased wheat to pea ratio. It was concluded by Adesogan *et al.* (2002) that the late cut, 1:3 pea:wheat ratio bi-crop silage had the highest nutritional quality when it was evaluated in feeding trials with sheep. However, in a study using dairy cows and the same silages, Salawu *et al.* (2002b) reported that cows fed the later cut, 3:1 pea:wheat bi-crop had the superior performance characteristics compared to the other bi-crop silages. The production of bi-crops of cereals and legumes calls, however, for a compromise in quality in either one or both of the component crops due to a difference in optimum harvest time, along with increased difficulty in chemical or biological control of pests and diseases (Anil *et al.*, 1998).

1.4 Condensed tannins

1.4.1 Overview of tannin biochemistry

Tannins are defined as any phenolic compound of high molecular weight containing many reactive phenolic hydroxyl (OH) or carboxyl (COOH) groups that enable them to complex with protein, minerals and other macromolecules (Reed, 1995). Tannins are water-soluble polyphenols that differ from other naturally occurring phenolic compounds in that they have the ability to precipitate proteins out of solution (Scalbert, 1991). Haslam (1974) concluded that the tannin protein interaction was due to the formation of hydrogen bonds. However, further work by Oh *et al.* (1980) has demonstrated that tannins can complex with proteins via hydrophobic bonding. Tannins can also bond covalently with proteins in the presence of the enzyme polyphenol oxidase (Reed, 1995). The complexes formed between tannins and proteins are pH dependent, and as a result are reversible (Aerts *et al.*, 1999). Jones and Mangan (1977) first identified that tannin protein complexes were stable between pH 3.5-7.0 and dissociated below pH 3.5, and they determined that the optimum pH for the complexing of CT and fraction 1 leaf protein (i.e. Rubisco) was pH 6.1. Reed (1995) reported that the strength of these complexes was affected by molecular weight, tertiary structure, isoelectric points and compatibility of the binding sites for both the tannin and protein.

Naturally occurring tannins have been classified into four groups (Handique and Baruah, 2002);

- Phloroglucinol derivatives
- Hydroxy cinnamic acid derivatives
- Galloyl and hexahydroxydiphenyl ester derivatives (or hydrolysable tannins, HYT)
- Proanthocyanidin derivatives (or CT)

The phloroglucinol derivatives are only found in brown marine algae (Handique and Baruah, 2002), and they will not be described any further. The hydroxy cinnamic acid derivatives are oligomers of mono lignols, and their most common form is lignin (Handique and Baruah, 2002). McDonald *et al.* (1998) reported that lignin complexes with many plant polysaccharides, including cellulose and cell wall proteins, rendering them indigestible by ruminant animals. The HYT have a carbohydrate core and occur mainly in the plant galls and fruit pods and rarely occur in forages (Min *et al.*, 2003). Min *et al.* (2003) reported that CT are distributed widely throughout the plant kingdom, most notably in woody browses and legumes. The CT consist of oligomers of flavan-3-ols (Figure 1.6) and the related flavanol residues (Mangan, 1988), linked through non hydrolysable interflavan carbon bonds (Reed, 1995) as shown in Figure 1.7.

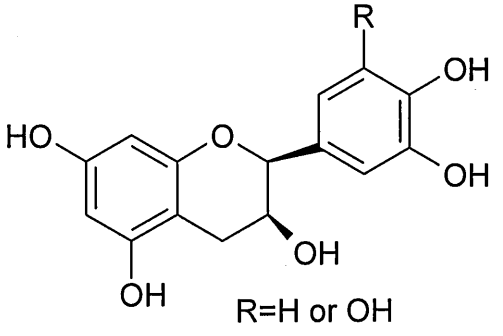


Figure 1.6 Chemical structure of Flavan-3-ol

Condensed tannins are secondary plant compounds that are stored in the vacuoles of plant cells (Aerts *et al.*, 1999) and play a role in the plant defensive mechanism, reducing infestation by pathogens (Aerts *et al.*, 1999), predation by herbivores (Robbins *et al.*, 1987a; 1987b) and acting as an antioxidant within plant cells (Carbonaro *et al.*, 1996).

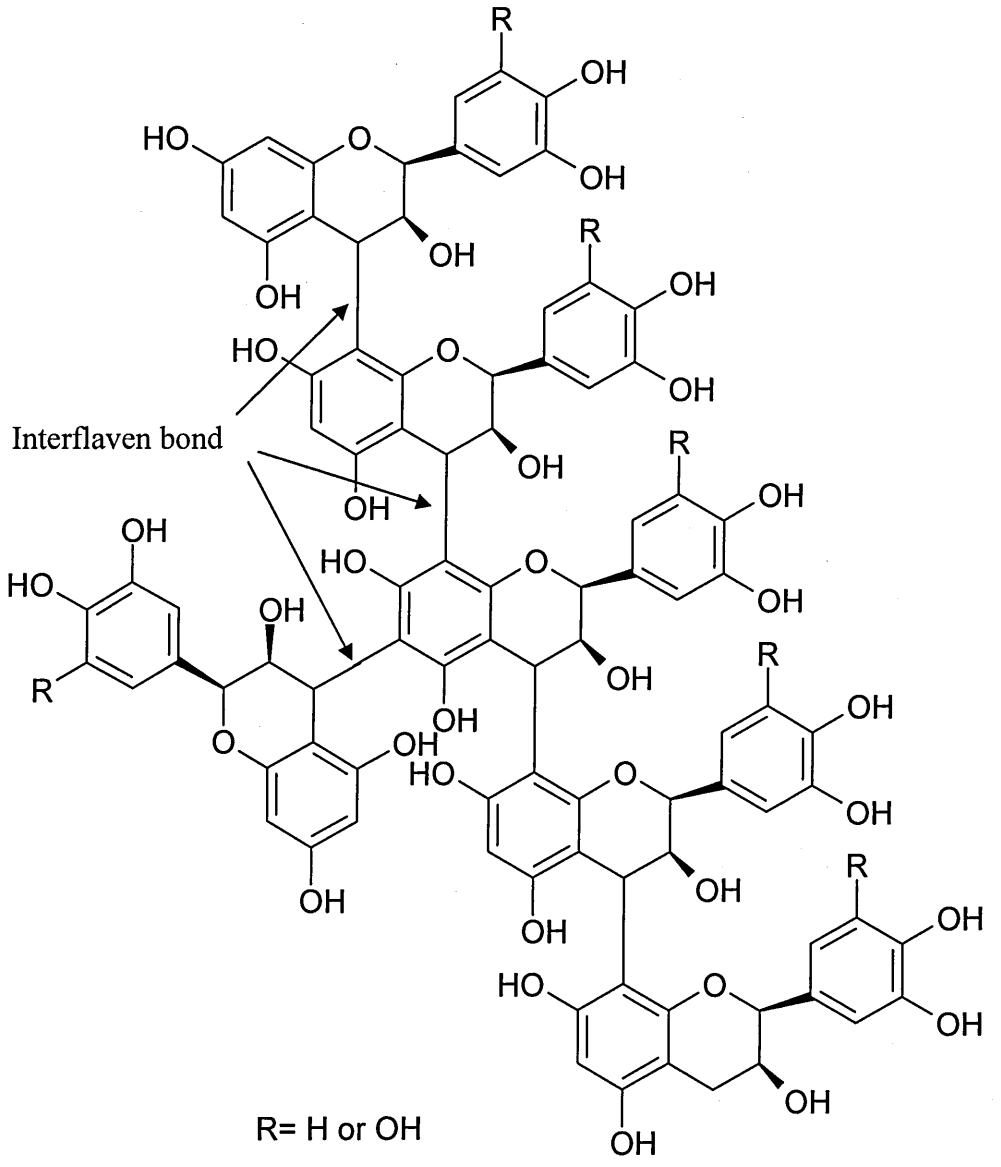


Figure 1.7 Chemical structure of a condensed tannin showing interflaven bonds. Adapted from Handique and Baruah (2002)

In tanniferious herbage, the CT can either be present as unbound, free CT, or bound to either protein or fibre (Terril *et al.*, 1992b). Table 1.12 summarises the distribution of CT in some temperate and tropical forage legumes. On average, the proportions of free CT, protein bound CT and fibre bound CT, are approximately 0.765, 0.190 and 0.045 of the total CT respectively in the forage legumes containing more than 2 g CT kg^{-1} DM.

Table 1.12 Condensed tannin (CT) content of some forage legumes

Forage legume		Condensed tannin (g kg^{-1} DM)			
Common name	Scientific name	Free CT	Protein-bound CT	Fibre-bound CT	Total CT
Big trefoil	*Lotus pedunculatus*	61	14	2	77
Birdsfoot trefoil	*Lotus corniculatus*	36	9	2	47
Lucerne	*Medicago sativa*	0	0.5	0	0.5
Red clover	*Trifolium pratense*	0.4	0.6	0.7	1.7
Sulla	*Hedysarum coronarium*	33	9	3	45

Adapted from Terril *et al.* (1992b)

Work by Minne *et al.* (2002) has demonstrated that the total amount of CT present in birdsfoot trefoil and sulla, before and after ensiling, remains constant, but the proportion of free CT reduced from 67% to 11% in birdsfoot trefoil and from 88% to 8% in sulla. This reduction in bio-availability from free CT to bound CT during ensiling was attributed by Minne *et al.* (2002) to the effect of plasmolysis, enabling the CT to complex with plant macromolecules, which in turn reduces protein breakdown.

1.4.2 Effects of condensed tannins on ruminants

The effect of tannins on the nutrition and health of ruminants has been the topic of a number or recent reviews including Aerts *et al.* (1999), Barry and McNabb (1999), McMahon *et al.* (2000) and Min *et al.* (2003).

A proposed CT interaction scheme in plant cells, the rumen and abomasum is presented in Figure 1.8. Mastication of forages ruptures the vacuoles and releases CT, initiating the binding of CT with both plant and salivary proteins forming agglutination of proteins instead of solubilisation (Waghorn and McNabb, 2003). Austin *et al.* (1989) demonstrated that the saliva of mule deer (*Odocoileus hemionus*) contained CT-binding proline-rich salivary proteins which reduced the effect of CT on dietary protein, whereas the salivary proteins of domesticated grazers, such as cattle and sheep, did not.

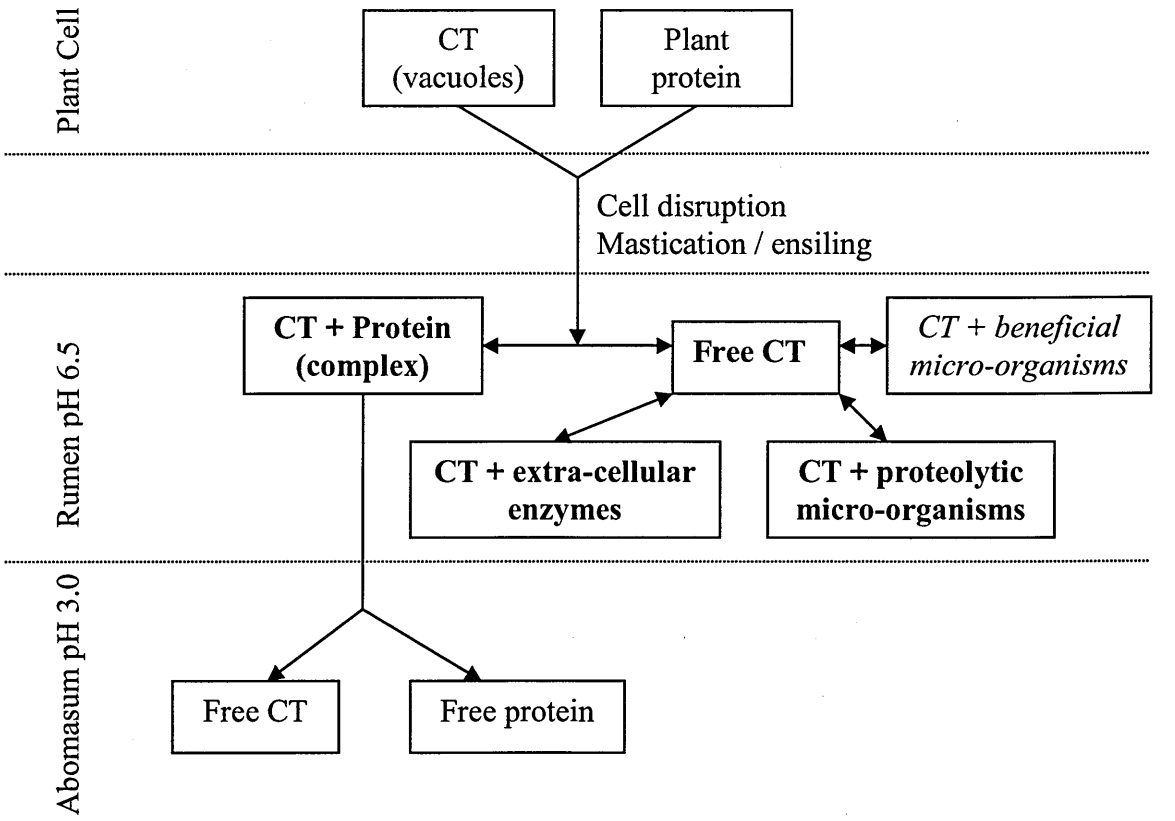


Figure 1.8 Proposed detrimental (red/italic) and beneficial (blue/bold) interactions of condensed tannin (CT) with protein and rumen micro-organisms. ↔ indicates a reversible reaction. Compiled with information from Mangan (1988) and Min *et al.* (2003)

The release of CT from plant cell vacuoles into solution in the rumen causes a reduction in protein availability (e.g. Robbins *et al.*, 1987a; Waghorn *et al.*, 1994b), a reduction in rumen micro-organism proteolytic activity (Min *et al.*, In press), a reduction in carbohydrate availability (Perez-Maldonado and Norton, 1996) and an increase in N efficiency (Broderick, 1995).

The presence of CT in ruminant diets can both detrimentally and beneficially affect ruminants in a number of ways (McLeod, 1974). Reviews by Aerts *et al.* (1999) and Barry and McNabb (1999) concluded that there was a dose dependent effect of CT, with low concentrations (20-40 g CT kg^{-1} DM intake) having a beneficial effect on protein turnover, and high concentrations (60-120 g CT kg^{-1} DM intake) affecting voluntary feed intake, essential nutrient availability and animal productivity. A summary of both beneficial and detrimental effects in sheep is displayed in Table 1.13.

Table 1.13 Nutritional effects of condensed tannin (CT) in sheep consuming forages

Forage plant	CT conc'n g kg^{-1} DM	Effect	Reference
Beneficial effects			
Lotus corniculatus	20	Increased absorption of essential amino acids	Waghorn *et al.* (1987)
Cercocarpus montanus	20-40	Reduced rumen ammonia concentration and increased fibre digestion	Nunez-Hernandez *et al.* (1991)
Lotus corniculatus	30	Increased absorption of methionine and cystine	Wang *et al.* (1996c)
Lotus corniculatus	30-40	Increased absorption of methionine and cystine	Wang *et al.* (1994)
Lotus corniculatus	30-40	Increased average daily live weight gain and wool growth rate	Wang *et al.* (1996b)
Hedysarum coronarium	40-50	Increased wool growth rate	Terril *et al.* (1992a)
Lotus corniculatus	40-50	Increased milk yield and milk protein percentage	Wang *et al.* (1996a)
Detrimental effects			
Leucaena leucocephala	10-20	Decreased dry matter degradability, decreased protein digestibility	Vitti *et al.* (2004)
Sesbania sesban	20-30		
Lotus pedunculatus	50-60	Reduced dry matter intake and reduced proportion of amino acids digested in the small intestine	Waghorn *et al.* (1994a)
Lotus pedunculatus	60	Reduced voluntary feed intake and reduced fibre digestion	Barry and Duncan (1984)
Lotus pedunculatus	80-90	Reduced lamb and wool growth	Barry (1985)
Lotus pedunculatus	90-100	Reduced fibre digestion	Barry *et al.* (1986)
Acacra aneura	120	Reduced dry matter intake and protein digestion	Pritchard *et al.* (1992)

Adapted from Aerts *et al.* (1999)

The results presented in Table 1.13 typically agree with the dose dependent effect of CT, with a forage CT content of 10 to 50 g kg^{-1} DM typically producing a beneficial effect and a higher level of 50 to 120 g CT kg^{-1} DM typically reducing ruminant performance. There are, however, exceptions from this general trend. Work by Vitti *et al.* (2004), with the Brazilian fodder legumes *Leucaena leucocephala* and *Sesbania sesban* containing between 10 and 30 g CT kg^{-1} DM, resulted in reduced dry matter intake and protein digestibility in sheep (Table 1.13), an effect potentially attributable to reduced soil fertility (Barry and Forss, 1983) caused by the low phosphorous content of Brazilian soils (Vitti *et al.*, 2004).

The CT-containing forages with beneficial effects that are presented in Table 1.13 are well suited to growth in temperate climates, whereas those with detrimental effects are more typically associated with growth in more arid climates (Min *et al.*, 2003).

Decreased palatability or negative effects on digestion may cause the reduction in dry matter intake of diets containing moderate to high levels of CT (Reed, 1995). Waghorn *et al.* (1994a) concluded that there was no difference between the rumen DM pool of sheep fed either CT containing forage with or without polyethylene glycol (a substance that binds irreversibly with CT) supplementation, suggesting that reduced intake is less associated with palatability and more associated with decreased rumen turnover. Waghorn and McNabb (2003) reported that dietary CT always increased the proportion (g kg^{-1} CP) of digestible undegradable plant protein that entered the abomasum.

Reed (1995) reported that when HYT are degraded, via hydrolysis reactions in the rumen, toxic products are formed, which might ultimately lead to death, but this was not the case with CT. The CT are not themselves toxic compounds, but they have acute effects on the digestion of carbohydrates and proteins (Reed, 1995). There is contradicting evidence on whether or not CT are digested, either ruminally or post ruminally. Terril *et al.* (1994) found no evidence to suggest that ^{14}C-labelled CT was absorbed in the gastrointestinal tract of sheep and work by Makkar *et al.* (1995) failed to show any degradation of CT *in vitro*, whereas Robbins *et al.* (1987a) concluded that, proportionally, only 0.60 of the tannin fed was recovered in the faeces of sheep, and Perez-Maldonado and Norton (1996) reported a proportional loss of CT in the rumen of 0.45 and a further 0.22 loss in the intestines. Barry and McNabb (1999) postulated that this difference might have been due to the differences in molecular weight, reactivity and chemical structure of the tannins utilised in the studies, with the largest more complex tannins being more resistant to degradation. Another possible explanation for the incomplete recovery of CT in faeces is the limitation of the standard acid-butanol method of Porter *et al.* (1986) to quantify fibre bound CT (Makkar *et al.*, 1999).

1.4.3 Condensed tannins and animal health

1.4.3.1 Anthelmintic effect

Aerts *et al.* (1999) reported that feeding CT to ruminants infected with intestinal nematodes had the potential to reduce nematode egg production. Recent work by Athanasiadou *et al.* (2000; 2001), Max *et al.* (2005) and Minho *et al.* (2005) has demonstrated a reduction in nematode egg output in faeces when increasing concentrations of CT were fed. However, Athanasiadou *et al.* (2001) failed to see a beneficial effect of feeding increased CT on total worm burden, suggesting that CT reduces the fecundity of nematodes, but does not remove the nematodes.

1.4.3.2 Reduction in bloat

Ruminants grazing swards rich in legumes are prevalent to bloat, which is caused by the rapid solublisation of Rubisco, causing a stable foam which is impermeable to gas, thus trapping it in the rumen and causing distension, potentially leading to death (Mangan, 1988). Chiquette *et al.* (1988) concluded that the stable protein foam causing bloat was markedly reduced by precipitation in legumes that contained CT, and subsequent work by Li *et al.* (1996) proposed that a minimum concentration of 5 g CT kg^{-1} DM conferred bloat safety. Waghorn and McNabb (2003) reported that there was no prevalence of bloat when ensiled legumes were fed, an effect that is potentially attributed to the reduction in Rubisco concentration during the early stages of ensilage.

1.5 Leguminous silages and animal production

1.5.1 Nutritional effects

Nitrogen based products entering the rumen from ensiled forages consist of PN, amino acid N, peptide N, amide N, ammonia N, nitrate N, nucleic acid N and dietary urea (Leng and Nolan, 1984; Givens and Rulquin, 2004), along with endogenous N. A diagrammatic representation of N metabolism in the rumen is displayed in Figure 1.9. The PN fraction of the forage can either be degradable or undegradable within the rumen (AFRC, 1992). Protein that is not rumen degradable passes into the abomasum, where it is exposed to enzymatic proteolysis and is either broken down and absorbed across the gut wall or excreted undigested in the faeces (Lapierre and Lobley, 2001).

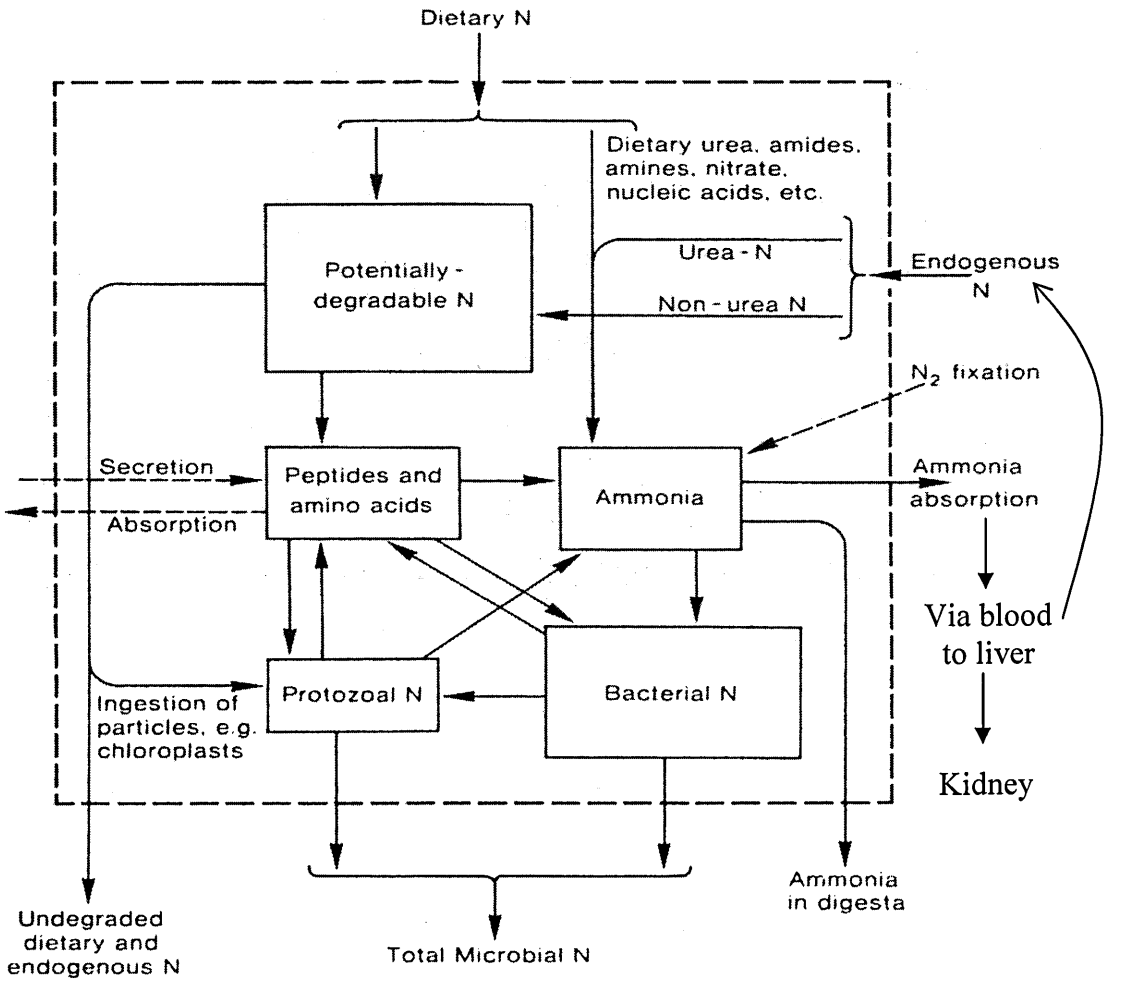


Figure 1.9 Metabolism of nitrogen in the rumen. Adapted from Leng and Nolan (1984)

Bach *et al.* (2005) stated that N metabolism in the rumen consists of two distinct events; firstly, protein degradation which provides a readily available N source, and secondly, microbial protein synthesis. The breakdown of PN to NPN in the rumen occurs through enzymatic proteolysis and can be restricted naturally by the presence of CT (Figure 1.6) or by the addition of anti-microbial compounds.

The rumen degradable protein and NPN sources are utilised by rumen micro-organisms as a substrate for the formation of microbial protein (Figure 1.10, Dewhurst *et al.*, 2000; Figure 1.10, Bach *et al.*, 2005), and not for growth, as most of the rumen bacteria are saccharolastic, relying on carbohydrates as a source of energy (Dehority, 2003). The formation of microbial protein requires a supply of N based substrate and energy, defined by AFRC (1992) as fermentable metabolisable energy (FME), from a carbohydrate source. If the energy source is limiting then amino acids are deaminated in the rumen and their carbon skeleton will be fermented into short chain volatile fatty acids (Figure 1.10, Bach *et al.*, 2005).

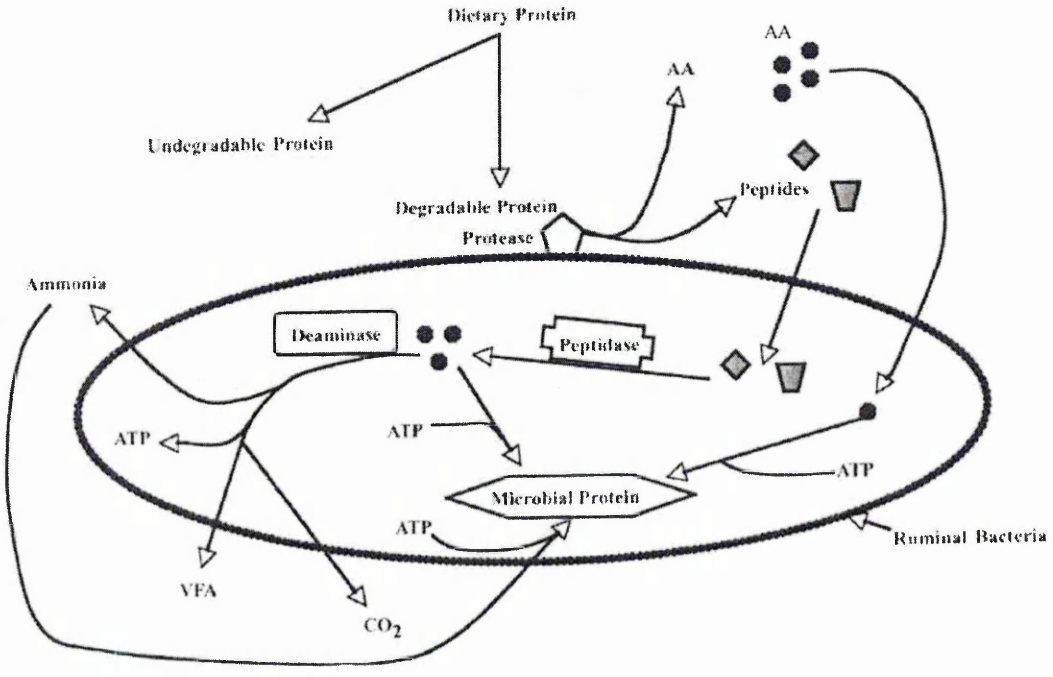


Figure 1.10 Diagrammatic representation of microbial protein synthesis within rumen bacteria. After Bach *et al.* (2005)

The use of the UK metabolisable protein system (AFRC, 1993) for formulating and balancing energy and protein supply within ruminant rations is based on an assumed yield of microbial crude protein per megajoule (MJ) of FME. It was proposed by AFRC (1992) that the yield of microbial crude protein (MCP) from FME should be calculated taking level of feeding (L, level x maintenance) into account, as follows:

$$\text{MCP yield g MJ}^{-1}\text{ FME (y)} = 7 + 6(1 - e^{(-0.35L)}) \qquad \text{Equation 1.1}$$

This resulted in MCP yields of 9, 10 and 11 g MJ^{-1} FME at feeding levels of 1, 2 and 3 respectively, which corresponded to rumen outflow rates of 0.02 h^{-1} (animal at maintenance), 0.05 h^{-1} (growing sheep and cattle) and 0.08 h^{-1} (lactating cattle and sheep) respectively (ARC, 1980). The ratio between effective rumen degradable protein (ERDP) and FME is a central part of diet formulation under AFRC (1993), since total MCP yield is based on the first limiting factor. If the ERDP:FME ratio is less than that of y (Equation 1.1), then ERDP is limiting and total MCP yield = ERDP. However, if the ERDP:FME ratio is greater than that of y, then FME is limiting and total MCP yield = y x FME. Diets formulated using AFRC (1993) should aim to provide an ERDP:FME ratio as close to y as possible, in order not to waste either or energy or protein.

In leguminous silages produced from perennial non-grain forming legumes, the residual water soluble carbohydrate content is low (<20 g kg^{-1} DM), as is the starch (<5 g kg^{-1} DM) content (Dewhurst *et al.*, 2000), and dietary supplementation with a carbohydrate source supplying more FME may be beneficial, by increasing ammonia capture by rumen micro-organisms (Leng and Nolan, 1984). *In vitro* work by Jaurena *et al.* (2005) has demonstrated that if red clover silage and grass silage are supplemented with barley, an increase in microbial protein synthesis is only observed in the red clover, reflecting an improved N capture by the rumen micro-organisms and a reduction in the rumen ammonia pool. Excess N in the form of ammonia can pass through the rumen wall and enter the

blood supply (Figure 1.9), where it is transported to the liver and converted to urea, which may be recycled back into the rumen (Lapierre and Lobley, 2001).

It was concluded by Storm and Ørskov (1983) that, proportionally, between 0.50 and 0.80 of the absorbed protein in the small intestines was of microbial origin. Rumen protozoa are unable to utilise rumen ammonia (Figure 1.9) but help regulate the level of soluble protein to sustain microbial growth (Bach *et al.*, 2005). Upon death, the majority of rumen protozoa lyse, releasing their cell contents back into the rumen, with a limited number flowing out of the rumen, and only contribute a small fraction (0.11 g g^{-1}) to total microbial N flow to the small intestines (Shabi *et al.*, 2000; Dehority, 2003).

Table 1.14 presents a list of *in sacco* rumen degradation characteristics and CP contents of grass and leguminous silages. On average, leguminous silages had a higher CP of 198 g kg^{-1} DM compared to 149 g kg^{-1} DM for grass silage, and a higher total potentially degradable N fraction (a+b) which relates to an increased supply in effective rumen degradable protein (ERDP) of 38 g kg^{-1} DM at an outflow rate of 0.08 h^{-1}. However, there was little difference in effective degradability (calculated at an outflow rate of 0.08 h^{-1}) between the grass and legume silages with values of 781 and 788 g kg^{-1} DM respectively. This increase in ERDP in leguminous silages, along with a lower residual WSC content compared to grass silage, tends to lead to an increase in rumen ammonia level and a lower N efficiency (Dewhurst *et al.*, 2000; Givens and Rulquin, 2004).

Table 1.14 Nitrogen degradability coefficients; immediately soluble N (a), potentially soluble N (b) and rate of degradation (c); CP contents, effective rumen degradable protein (ERDP) and effective degradability (ED) of grass and whole-crop leguminous silages derived from the model of Ørskov and McDonald (1979).

Silage	CP (g kg^{-1} DM)	a (kg kg^{-1})	b (kg kg^{-1})	c (h^{-1})	n†	ERDP‡ (g kg^{-1} DM)	ED‡ (kg kg^{-1})	Reference
Grass	157	0.625	0.255	0.160	2	105	0.795	1
	140	0.680	0.217	0.031	1	85	0.741	2
	149	**0.653**	**0.236**	**0.096**		**95**	**0.768**	Mean
Red Clover	196	0.582	0.400	0.046	1	120	0.728	2
White clover	261	0.687	0.248	0.063	1	172	0.796	2
Peas	170	0.785	0.159	0.097	1	122	0.872	4
	188	0.769	0.165	0.119	3	134	0.868	5
	178	0.703	0.220	0.077	1	119	0.811	6
	179	**0.752**	**0.181**	**0.098**		**125**	**0.850**	Mean
Pea/wheat	178	0.662	0.197	0.078	5	112	0.759	7
Lucerne	194	0.660	0.250	0.170	1	135	0.830	1
	199	0.600	0.332	0.203	4	143	0.838	3
	193	0.785	0.149	0.090	1	136	0.864	4
	195	**0.682**	**0.244**	**0.154**		**138**	**0.844**	Mean
Beans	222	0.494	0.415	0.132	1	145	0.752	6

†number of samples averaged. ‡ calculated at a rumen outflow rate of 0.08 h^{-1}. Compiled from 1 AFRC (1993), 2 Dewhurst *et al.* (2003a), 3 Hristov and Sandev (1998), 4 Mustafa *et al.* (2000), 5 Mustafa *et al.* (2002), 6 Mustafa and Seguin (2003) and 7 Salawu *et al.* (2001b)

1.5.1.1 Nitrogen balance

Concern has been expressed over the inefficiency of N utilisation in silage based diets, and it was concluded that a greater understanding of the interaction between carbohydrate and N was needed (Givens and Rulquin, (2004). The proportion of N and energy supplied from grass silage is unbalanced with an effective rumen degradable protein to fermentable metabolisable energy ratio of 6.7 (AFRC, 1993), leading to inefficient use of dietary N. Low efficiency of N utilisation is indicative of an excess of rumen degradable protein in relation to available utilisable carbohydrate supply (Givens and Rulquin, 2004), whereas apparent whole-tract digestibility of N incorporates N metabolised through the kidneys and excreted in the form of urine (Lapierre and Lobley, 2001). Recent examples of the N

balance of wether lambs fed ensiled whole-crop leguminous silages *ad libitum* are presented in Table 1.15.

Table 1.15 Nitrogen balance of wether lambs fed ensiled grass or legumes *ad libitum* without supplementary concentrate. Values in parentheses represent proportion of intake (kg kg^{-1})

	N intake g d^{-1}	Faecal N g d^{-1}	Urinary N g d^{-1}	N retained g d^{-1}	Apparent digestibility of N (g g^{-1})
Rye grass[1]	14.7	6.4 (0.435)	6.4 (0.435)	1.9 (0.129)	0.565
Red clover[2]	51.0	16.5 (0.324)	24.5 (0.480)	10.0 (0.196)	0.676
Lucerne[2]	38.5	12.0 (0.312)	26.0 (0.675)	0.5 (0.013)	0.688
Lotus[2]	55.0	15.0 (0.273)	24.0 (0.436)	16.0 (0.291)	0.727
Sainfoin[2]	24.0	20.0 (0.833)	7.0 (0.292)	-3.0 (-0.125)	0.166
Peas[3]	27.0	7.5 (0.278)	13.5 (0.500)	6.0 (0.222)	0.722
Beans[3]	28.0	9.5 (0.339)	15.5 (0.554)	3.0 (0.107)	0.661
Pea/wheat[1]	30.5	11.4 (0.374)	12.3 (0.403)	6.8 (0.223)	0.626

Compiled from [1] Adesogan *et al.* (2002), [2] Fraser *et al.* (2000) and [3] Fraser *et al.* (2001)

Lambs consuming any of the leguminous silages had an increased N intake compared to grass, related to the higher CP content of the forages. However, only those lambs fed red clover, lotus, peas and pea/wheat bi-crops had a higher proportion of N retained compared to grass silage which may indicate that there was an increased energy supply from these forages in relation to grass silage. The low proportion of N retained in the lambs fed the lucerne or bean silage may be related to the low residual water soluble carbohydrate content of the silages. The water soluble carbohydrate content of the rye grass, red clover, lucerne, lotus, sainfoin, peas, beans and pea/wheat bi-crops were 7.8, 9.1, 5.8, 49.9, 29.3, 42.0, 14.2 and 18.2 g kg^{-1} DM respectively. Fraser *et al.* (2000) attributed the negative N balance observed in lambs fed sainfoin silage to the high level of condensed tannin present in this crop, but the level of tannin was not stated. This conclusion was derived from the observation that the proportion of feed N excreted in the faeces was 0.833 kg kg^{-1}, an indication of decreased rumen N metabolism caused by the protein binding action of condensed tannins.

Nitrogen balance data for cattle consuming leguminous forages *ad libitum* without concentrate supplementation is scarce. However, work by Adesogan *et al.* (2004) examined the intake and N balance of dry dairy cows consuming grass silage or pea/wheat bi-crops differing in CT content, and their results are summarised in Table 1.16. The total amount of consumed CT was similar between the magnus/wheat and setchey/wheat bi-crops. However, the amount of free CT was 15.6 g quebracho tannin equivalent d^{-1} higher in the setchey/wheat bi-crop and the difference in the proportion of N retained and apparent whole-tract digestibility of N can be attributed to the increased level of free CT.

Table 1.16 Nitrogen balance of dry dairy cows fed grass silage or pea/wheat bi-crops *ad libitum* differing in condensed tannin content (CT)

	Magnus/wheat	Setchey/wheat	Grass silage
DM intake kg d^{-1}	12.0	12.0	7.4
N intake g d^{-1}	340	320	220
Urinary N g d^{-1}	115	116	122
Faecal N g d^{-1}	72	90	87
N retained g d^{-1}	153	114	11
N retention kg kg^{-1}	0.450	0.356	0.050
Apparent N digestibility kg kg^{-1}	0.788	0.719	0.605
Free CT intake† g d^{-1}	33.6	49.2	NM
Fibre bound CT† g d^{-1}	64.8	54.0	NM
Total CT intake† g d^{-1}	98.4	103.2	NM

† equivalent to quebracho tannin. NM not measured. Adapted from Adesogan *et al.* (2004)

Nitrogen efficiency data of dairy cows fed leguminous silages *ad libitum* with the same concentrate formulation fed to the control group is limited. However, recent examples are presented in Table 1.17.

Table 1.17 Nitrogen efficiency for milk production (kg kg^{-1}) of dairy cows consuming grass or leguminous silage *ad libitum* supplemented with concentrate (Conc.)

	Conc. kg d^{-1}	N intake g d^{-1}	Milk N g d^{-1}	N efficiency for milk production	Reference
Grass	6	406	99	0.244	Salawu *et al.* (2002a)
Pea/wheat	6	497	107	0.215	
Grass	8	555	141	0.254	Bertilsson *et al.* (2001)
Red clover	8	699	146	0.209	
White clover	8	780	160	0.205	
Galega	8	765	143	0.187	
Lucerne	8	823	149	0.181	
Grass	8	551	130	0.236	Dewhurst *et al.* (2003b)
Red clover	8	711	140	0.197	
White clover	8	809	164	0.203	

Typically, cows fed leguminous silages *ad libitum* had lower N efficiencies compared to animals fed the grass silage control, which may be attributed to the same reasons described for lambs i.e. excess rumen degradable protein and a lack of sufficient fermentable metabolisable energy. Nitrogen efficiency for milk production can be increased in leguminous silages by feeding a balanced ration. Mustafa *et al.* (2000) reported N efficiencies for milk production of 0.286 and 0.284 g g^{-1} for high yielding (45 kg milk d^{-1}) dairy cows fed pea silage and lucerne silage respectively mixed 50:50 on a DM basis with a concentrate, in a total mixed ration balanced for energy and protein.

There are very few examples of forages differing in CT content and N efficiency for milk production. Work by Hymes-Fecht *et al.* (2004), with normal, low and high CT varieties of birdsfoot trefoil, reported relative N efficiencies for milk production of 0.829 and 0.947 for the low and high CT varieties compared to the normal CT variety. Work by Adesogan *et al.* (2004), with pea/wheat bi-crops differing in CT content, indicated that that cows consuming the equivalent of 136 g quebracho tannin d^{-1} had a higher N efficiency for milk production of 0.216 compared to 0.197 g g^{-1} for cows consuming the equivalent of 115 g quebracho tannin d^{-1}.

1.5.1.2 Rumen volatile fatty acid production

The effect of feeding leguminous silages on rumen volatile fatty acid (VFA) production is presented in Table 1.18.

Table 1.18 Relative proportions of rumen volatile fatty acids (VFA) of dairy cattle fed leguminous silages *ad libitum* related to the grass silage control. (all mmol l^{-1}) Values in parentheses represent molar proportions of VFAs measured

	Grass/red clover†	Red clover	Grass/white clover†	White clover	Lucerne	Pea/wheat
Acetic	1.02 (0.674)	1.02 (0.676)	1.00 (0.660)	0.98 (0.648)	1.00 (0.665)	1.07 (0.659)
Propinonic	0.91 (0.181)	0.93 (0.186)	0.95 (0.189)	0.98 (0.195)	0.89 (0.177)	0.72 (0.205)
iso-Butyric	1.11 (0.010)	1.05 (0.010)	1.33 (0.012)	1.33 (0.012)	1.45 (0.013)	1.23 (0.012)
n-Butyric	1.04 (0.110)	1.00 (0.106)	1.01 (0.107)	1.08 (0.115)	1.07 (0.113)	1.07 (0.091)
iso-Valeric	0.96 (0.011)	0.96 (0.011)	1.46 (0.016)	1.45 (0.016)	1.36 (0.015)	1.16 (0.020)
n-Valeric	1.08 (0.012)	1.08 (0.013)	0.34 (0.016)	1.41 (0.012)	1.27 (0.015)	0.93 (0.014)
Total VFA	80.5	94.1	83.1	107.3	93.8	71.9
Reference	1	1	1	1	1	2

† grass and clover mixed 50:50 on a DM basis. Adapted from 1 Dewhurst *et al.* (2003a) and 2 Adesogan *et al.* (2004)

Total rumen VFA concentration tended to be higher in cows fed the leguminous silages than the mean of animals fed the grass silage control of 79.3 mmol l^{-1}, except for the animals consuming the pea/wheat bi-crop which had a lower total VFA concentration of 71.9 mmol l^{-1}. On average, feeding leguminous silages or a 50:50 mix of grass silage and legume silage altered the proportions of acetate, propionate and n-butyrate by 1.01, 0.90 and 1.07 fold respectively, in relation to the respective grass silage control. The decrease in the proportion of ruminal propionate of cattle fed ensiled leguminous forages was the same as the decrease of 0.90 observed by Beever *et al.*(1987) with fresh white clover herbage, in comparison to the fresh grass control. The higher proportions observed for iso-butyrate, iso-valerate and n-valerate of 1.22, 1.20 and 1.22 fold respectively, only represented a very small change in concentration of less than 1 mmol l^{-1} and were attributed by Dewhurst *et*

al. (2003a) to an excess of rumen degradable protein. The mean acetate to propionate to buyrate (iso + n) ratio observed in Table 1.18 for the leguminous silages and the grass/legume silage mixes was 69.2:18.5:12.4, which when compared to the mean grass silage control of 70.7:17.4:11.9 represents very little difference.

1.5.2 Animal performance

There have been a number of studies with both sheep and dairy cattle that have evaluated the complete replacement of grass silage with whole-crop leguminous silages, and these are summarised in Table 1.19. The general effect of replacing grass silage with leguminous silage was to increase the average forage dry matter intake by 1.18 fold, increase growth rate by 1.08 fold (in sheep), increase milk production by 1.09 fold, increase fat yield by 1.06 fold and to increase protein yield by 1.08 fold.

1.5.2.1 Intake

The increase in forage dry matter intake of ruminants consuming leguminous forages compared to grass has partly been attributed to the greater particle fragility of legumes (Waghorn *et al.*, 1989) resulting in an increased outflow from the reticulo-rumen (Allen, 2000). It was concluded by Waghorn *et al.* (1989) that forage particles less than 2 mm in diameter flowed freely out of the reticulo-rumen. In their study, Waghorn *et al.* (1989) examined the extent of particle breakdown of fresh ryegrass and fresh lucerne in the rumen, and concluded that ryegrass particles decreased in size slower and therefore remained in the reticulo-rumen longer than particles from lucerne. The same effect was seen by Dewhurst *et al.* (2003a), with ensiled legumes, with a mean of 407 g kg^{-1} DM of ensiled ryegrass particles being retained on a 2 mm sieve compared to a mean of only 337 g kg^{-1} DM in lucerne. Broderick *et al.* (2002) and Hoffman *et al.* (1998) reported an

Table 1.19 Relative performance of sheep and dairy cattle fed whole-crop leguminous silages relative to animals fed grass silage (kg kg^{-1}). Values in parentheses represent actual intakes, growth rates and yields (kg d^{-1})

Silage	Species	FDMI	ADLWG	Milk yield	Fat yield	Protein yield	Reference
Red clover	Sheep	1.38 (0.68)	1.54 (0.11)				Speijers *et al.* (2005)
	Cattle	1.08 (13.6)		1.05 (29.7)	1.01 (1.27)	1.03 (0.83)	Bertilsson *et al.* (2001)
	Cattle	1.19 (12.1)		1.01 (19.9)	0.98 (0.80)	0.98 (0.64)	Dewhurst *et al.* (2003a)
	Cattle	1.18 (13.4)		1.13 (28.1)	1.17 (1.29)	1.09 (0.88)	Dewhurst *et al.* (2003b)
White clover	Cattle	1.05 (13.2)		1.14 (32.1)	1.06 (1.29)	1.13 (1.02)	Bertilsson *et al.* (2001)
	Cattle	1.33 (13.6)		1.22 (20.5)	1.19 (0.98)	1.20 (0.79)	Dewhurst *et al.* (2003a)
	Cattle	1.13 (12.9)		1.27 (31.5)	1.27 (1.40)	1.25 (1.01)	Dewhurst *et al.* (2003b)
Pea/wheat	Cattle	1.23 (16.1)		1.08 (22.4)	0.99 (1.05)	1.08 (0.68)	Salawu *et al.* (2002a)
	Cattle	1.26 (14.9)		1.11 (22.4)	1.15 (0.96)	1.14 (0.70)	Adesogan *et al.* (2004)
Galega	Cattle	1.06 (12.5)		1.00 (28.4)	0.98 (1.23)	1.01 (0.91)	Bertilsson *et al.* (2001)
Lucerne	Sheep	1.14 (0.66)	0.61 (0.05)				Speijers *et al.* (2005)
	Cattle	1.11 (15.3)		1.05 (31.8)	1.01 (1.14)	1.06 (0.93)	Hoffman *et al.* (1998)
	Cattle	1.18 (15.0)		1.04 (29.5)	0.98 (1.23)	1.06 (0.95)	Bertilsson *et al.* (2001)
	Cattle	1.22 (12.4)		0.98 (19.3)	0.94 (0.78)	0.96 (0.63)	Dewhurst *et al.* (2003a)
	Cattle	1.19 (13.6)		1.11 (27.7)	1.10 (1.21)	1.10 (0.89)	Dewhurst *et al.* (2003b)

increased dry matter intake of dairy cows consuming lucerne silage compared to those fed ryegrass silage, despite the fact that the ryegrass, in both studies, had a significantly higher apparent DM and NDF digestibility. In a rumen evacuation study by Dewhurst *et al.* (2003a) it was reported that the digestion kinetics of grass silage were slowed by a persistent mat, which formed in the rumen of cows offered this feedstuff in relation to lucerne. In studies by Broderick (1985) and Colenbrander *et al.* (1986), feeding lucerne or maize silage, and Mustafa *et al.* (2000), feeding lucerne or barley silages, similar forage dry matter intakes were observed in dairy cattle. It has been postulated by Allen (2000) that the greater gut fill effect caused by grass (mono-cotyledons) NDF compared to legume NDF is limited only to ryegrass.

1.5.2.2 Growth and milk production

The higher CP content (Table 1.14) and higher predicted ME content (Speijers *et al.*, 2005) of leguminous silages, coupled with increased forage dry matter intakes, typically results in animals consuming whole-crop leguminous silages having increased growth or increased milk production compared to those fed on ryegrass silage (Table 1.19). There is, however, no predictive equation for ME content of ensiled legumes (AFRC, 1993). The use of the equation formulated by Barber *et al.* (1989), based on organic matter digestibility, has been shown by Speijers *et al.* (2005) to overestimate ME supply in ensiled lucerne based on lamb performance. However, use of the same equation matched the performance (related to ME supply) of lambs fed both ensiled red clover and ryegrass. Broderick (1995) concluded that ruminants fed diets containing lucerne should be supplemented with a source of fermentable carbohydrate or an increased level of digestible undegradable protein in order to increase feed conversion efficiency and hence milk yield.

It has been concluded by Hristov *et al.* (2004) that milk yield is positively correlated with forage dry matter intake. It is plausible to suggest that the increases reported in milk fat

and protein yield are related to increased ruminal acetate production (Table 1.18) and the higher protein content of the leguminous forages compared to grass silage (Table 1.14). The forage dry matter intake observed in the pea/wheat bi-crops (Table 1.19) may be attributable to the effect of mixing forages as described by Phipps *et al.* (1995). However, they are comparable to the monoculture leguminous silages. There is little information on the effect of inclusion rate of whole-crop leguminous silages in ruminant diets, with many studies evaluating the total replacement of one type of forage with another. Work by Dewhurst *et al.* (2005) has shown that feeding forage mixtures of red clover and maize silage (60:40 DM basis) *ab libitum* resulted in an increased forage dry matter intake and milk yield of 1.50 fold and 1.32 fold respectively, compared to grass silage *ad libitum* relating to a 5 kg d^{-1} increase in milk output. However, an increased milk yield of 4.2 kg d^{-1} was achieved by restricting the forage dry matter intake of the clover/maize mix to the same level as the cows fed grass silage, suggesting that dairy cattle consuming leguminous silages do not reach a state of satiety due to an increased whole-tract passage rate of leguminous silages and are only limited by their ability to metabolise absorbed nutrients (Illius and Jessop, 1996). The inclusion of whole-crop red clover silage and maize silage mixed 25:75 (DM basis) intake resulted in an increased milk yield and N efficiency for milk production compared to animals fed the 40:60 mix (Dewhurst *et al.*, 2005), demonstrating that ration balancing is essential with diets containing whole-crop leguminous silages.

1.6 Conclusions from literature review

- Imported soya-bean meal forms a major constituent of the protein requirements of ruminant livestock within the UK
- The production of home-grown rapeseed meal is limited by the low subsidy and restrictions imposed on the EU by the Blair House Agreement
- The annual legumes peas and beans are well suited to the UK climate and offer the potential for use as a whole-crop silage, in conventional and organic systems, due to their N fixing ability
- Ensiling of whole-crop legumes is possible, but little information exists on the optimum conditions for harvesting annual legumes for silage
- The presence of condensed tannin at a level of 10-50 g kg^{-1} dry matter intake confers bloat prevention and typically has beneficial effects on protecting plant protein during ensiling and ruminal degradation.
- Leguminous silages have high levels of effective rumen degradable protein, and as a result, are utilised inefficiently in the rumen
- Feeding whole-crop leguminous silages to ruminants increases forage dry matter intake, growth rate, milk production and milk component yield compared to grass silage as the sole forage

1.7 Experimental objectives

The objective of this study is to evaluate the optimum conditions for the production of whole-crop silages, from the annual grain forming legumes, peas and beans, that differ in condensed tannin content and are harvested at the recommended growth stages of 205 and 207 respectively. The silages produced from the field scale trials will be evaluated as a potential replacement to soya-bean meal and their effects on nutrient utilisation and performance of finishing lambs and performance of dairy cows evaluated.

2.0 GENERAL MATERIAL AND METHODS

2.1 Dry matter

Sub-samples of silage, concentrate, residues from synthetic bags and faeces were dried to a constant weight in a forced draught oven at 65°C unless otherwise stated. Dry matter was calculated as

$$\text{Dry matter (g kg}^{-1}) = \left(\frac{\text{dried sample weight (g)}}{\text{initial sample weight (g)}} \right) \times 1000 \qquad \text{Equation 2.1}$$

2.2 Ash

Ash was determined by an adaptation of AOAC (2000). Approximately 2 g of dried sample was weighed into a pre-weighed porcelain crucible and heated to 500°C in a muffle furnace (Gallenkamp, Size 3) for 16 h. Samples were then cooled in a desiccator and reweighed.

$$\text{ash (g kg}^{-1}\text{ DM}) = \left(\frac{\text{weight of ash (g)}}{\text{sample weight (g)}} \right) \times 1000 \qquad \text{Equation 2.2}$$

Organic matter (g kg^{-1} DM) was calculated as 1000 - ash.

2.3 Crude protein

Nitrogen was determined by steam distillation as described by the method of FOSS (2002). Exactly 5 ml of urine or approximately 2 g of dried ground sample was accurately weighed onto a filter paper (90 mm diameter Whatman No1, Maidstone, UK) and transferred into a 250 ml digestion tube, to which two kjeltabs CK (3.5 g potassium sulphate and 0.4 g

copper (II) sulphate per tablet, Thompson and Capper, Runcorn, UK) were added. Exactly 16 ml of concentrated (0.98 mass mass^{-1}) sulphuric acid (Analar, VWR, Lutterworth, UK) was added to each tube. Samples were then refluxed at 400°C for 45 min and allowed to cool for 10 min prior to the addition of 75 ml of cold distilled water. Nitrogen was determined by back titration using 0.2 M hydrochloric acid (Covalar, VWR, Lutterworth, UK) as the titrant by an auto-analyser (FOSS, Warrington, UK). Crude protein was determined as

$$\text{Crude protein}_{(g\ kg^{-1}\ DM)} = \text{total nitrogen}_{(g\ kg^{-1}\ DM)} \times 6.25 \qquad \text{Equation 2.3}$$

2.4 Ammonia nitrogen

Ammonia nitrogen was determined by an adaptation of MAFF (1986). Approximately 20 g of fresh forage was accurately weighed into a 200 ml glass shaking bottle, to which 100 ml of distilled water was added. Bottles were capped and placed on a laboratory shaker for 1 h (275 strokes per min). The solution was then filtered through a 150 mm Whatman number 1 filter paper. Exactly 10 ml of filtrate was pipetted into a 250 ml kjeldahl digestion tube to which 6 ml of magnesium hydroxide solution (17 g of ignited heavy magnesium oxide (VWR, Lutterworth, UK) suspended in 100 ml of distilled water) was added. The sample was steam distilled, using an auto-titrator (FOSS 1030 auto-titrator, FOSS, Warrington, UK) and the liberated ammonia was bubbled through 25 ml of receiver solution. Receiver solution was made by dissolving 50 g of boric acid in 5 l of distilled water, 50 ml of bromocresol green (100 mg in 100 ml methanol), 35 ml of methyl red solution (100 mg in 100 ml methanol), and 1.5 ml of 0.1 M sodium hydroxide solution was added. The receiver solution was then back titrated using 0.01 M hydrochloric acid (VWR, UK) as the titrant. Ammonia nitrogen was determined as

$$\text{Ammonia nitrogen (g kg}^{-1}\text{ DM)} = \left(\frac{7 \times T \times (120 - 0.02DM)}{10 \times DM}\right) \quad \text{Equation 2.4}$$

Where T is titre reading (corrected for method blank) and DM (g kg^{-1}) is the dry matter of the silage. Ammonia nitrogen was also expressed as g kg^{-1} total nitrogen (TN) content.

2.5 pH

The pH of the fresh forages was determined by the method of MAFF (1986). Approximately 50 g fresh forage was weighed into a 250 ml beaker, to which 100 ml distilled water was added. The beaker was swirled 5 times every 15 min for 1 h. The solution was filtered through a filter paper (Whatman No. 1, Maidstone, UK) and the pH of the filtrate was determined using a Russell LS120 pH meter. The probe was washed in distilled water between every sample and was calibrated using pH 4 and 7 standards (colourkey, VWR, Lutterworth, UK) every 10 samples.

2.6 Neutral detergent fibre

Neutral detergent fibre (NDF) was determined by an adaptation of the method of Goering and Van Soest (1979). Approximately 0.5 g of feed or faeces was accurately weighed into a sintered glass crucible (porosity 1, Soham Scientific, Ely, UK). Crucibles were placed into Fibertec apparatus (1020, FOSS, Warrington, UK) and washed with 3 x 25 ml of petroleum spirit (GPR, VWR, Lutterworth, UK) under vacuum. Exactly 25 ml of neutral detergent reagent (made by dissolving 93 g of disodium ethylene diamine tetra-acetae dihydrate and 34 g of sodium borate in 3 l of distilled water using gentle heat. To this, 150 g of sodium lauryl sulphate and 50 ml of 2-ethoxy ethanol was added. In a separate flask 22.8 g of anhydrous disodium hydrogen phospahate was dissolved in distilled water. The two solutions were then mixed and diluted to 5 l. The pH was adjusted to lie between 6.9

and 7.1 (with either 0.1 M NaOH or 0.1 M HCl) and 0.5 ml of octan-1-ol (FOSS, Warrington, UK) was added to each sample.

Samples were boiled and refluxed for 30 min, after which an additional 25 ml of cold neutral detergent reagent and 2 ml of α-amylase (2 g of heat stable α-amylase E.C.3.2.1.1. from *Bacillus subtilis* (Sigma, Gillingham, UK) dissolved in 90 ml of distilled water and 10 ml of 2-ethoxy ethanol) was added. Samples were boiled and refluxed for a further 30 min, drained and then washed with 3 x 25 ml of hot (80°C) water under vacuum. To each sample, 25 ml of hot (80°C) water and 2 ml of α-amylase solution was added. After 15 min the samples were drained and washed, under vacuum, with 3 x 25 ml of hot (80°C) water and once with 25 ml propanone. Crucibles were removed from the Fibretec apparatus and dried at 100°C overnight. After cooling in a desiccator, crucibles were weighed and then placed in a muffle furnace at 500°C for 16 h. Crucibles were then allowed to cool to room temperature in a desiccator and weighed. NDF (g kg^{-1} DM) was calculated as

$$\text{NDF (g kg}^{-1}\text{DM)} = \left(\frac{\text{residue weight (g) - ash weight (g)}}{\text{sample weight (g DM)}} \right) \times 1000 \qquad \text{Equation 2.5}$$

2.7 Neutral cellulase plus gamanase digestibility

Neutral cellulase plus gamanase digestibility (NCDG) was determined by the method of MAFF (1993) using Fibretec apparatus (1020, FOSS, Warrington, UK). The method commenced in the same way as for NDF determination, and continued up until the propanone wash. Crucibles were removed from the Fibretec apparatus and the bottoms plugged with a subaseal. To each crucible, 25 ml of buffered cellulase and gamanase solution was added. The buffer consisted of 1.36 g sodium acetate (Analar, VWR, Lutterworth, UK) dissolved in 500 ml distilled water to which 0.6 ml glacial acetic acid was added and the volume made up to 1 l, and the pH adjusted to 4.8. Exactly 20 g

cellulase (from *Aspergillus niger*, Sigma Aldrich, Gillingham, UK) and 0.1 g chloramephenicol (Sigma, Gillingham, UK) was added to 1 l of buffer, and the solution was mixed and incubated at 40°C for at least 1 h prior to use. Exactly 900 ml of buffered cellulase was mixed with 100 ml of gamanase solution (Novozyme, Netherlands).

The crucibles were placed in an incubator maintained at 40°C for 16 h. The subaseals were removed and the crucibles placed back in the Fibretec apparatus. The samples were washed under vacuum with 3 x 20 ml of hot (80°C) water and once with 25 ml propanone. The crucibles were dried overnight at 100°C and weighed, after cooling in a desiccator, prior to being ashed at 550°C for 4 h. The samples were allowed to cool in a desiccator and re-weighed. Neutral cellualse plus gamanase digestibility was calculated as follows

NCDG (g kg^{-1} DM) = 1000 – (indigestible organic matter (g kg^{-1}) + ash (g kg^{-1})) Equation 2.6

2.8 Starch

Starch content of feed samples was determined by the method of Rasmussen and Henry (1990) after removing soluble sugars using the method of Henry (1985). Approximately 50 mg was accurately weighed into a screw topped culture tube (16 x 100 mm). To each tube exactly 3 ml of 800 g l^{-1} ethanol (Analar, VWR, Lutterworth, UK) was added, and the tube capped with a PTFE lined cap. The contents were mixed vigorously on a vortex mixer for 30 seconds and the tubes were then placed in a water bath maintained at 80°C for 10 min, prior to being centrifuged at 2000 xg for 5 min. The supernatant was removed and discarded. The sample was then washed centrifugally with 3 ml 800 g l^{-1} ethanol, and the supernatant discarded.

Exactly 2.5 ml of acetate buffer (148 ml of 0.2 M acetic acid (Analar, VWR, Lutterworth, UK) added to 352 ml of 0.2 M sodium acetate (Analar, VWR, Lutterworth, UK), made up to 1 l with distilled water and adjusted to pH 5.0) was added to each tube along with 20 µl

Teramyl (α amylase, EC 3.2.1.1, 5,000,000 units ml^{-1}, Sigma Aldrich, Gillingham, UK). The tube was capped and mixed for 30 seconds on a vortex mixer and placed in a boiling water bath for 30 min. During this time the tubes were vortex mixed three times. The samples were then allowed to cool to room temperature and exactly 10 µl of amyglucosidase (EC 3.2.1.3 from *Aspergillus niger*, Sigma Aldrich, Gillingham, UK) was added to each sample. The tubes were loosely capped and placed in an incubator maintained at 60°C for 16 h. The samples were centrifuged at 2200 xg for 15 min and exactly 0.5 ml of supernatant was diluted to 10 ml with distilled water in a test tube. Exactly 200 µl was transferred to a clean glass test tube (15 x 100 mm) and exactly 5 ml glucose oxidase solution was added. Glucose oxidase solution was prepared by dissolving 24.8 g disodium hydrogen orthophosphate (Analar, VWR, Lutterworth, UK), 12.4 g sodium dihydrogen orthophosphate (Analer, VWR, Lutterworth, UK), 4.0 g benzoic acid (dispersed in a 0.1 ml ethanol, GPR, VWR, Lutterworth, UK), 0.2 g 4-amino-antipyrine (Sigma Aldrich, Gillingham, UK) and 3.0 g p-hydroxybenzoic acid (Sigma, Gillingham, UK) into 1800 ml distilled water. To this, 40 mg glucose oxidase (EC 1.1.3.4 from *Aspergillus niger*, Sigma Aldrich, Gillingham, UK) and 10 mg peroxidase (EC 1.11.1.7 from horse radish, Sigma Aldrich, Gillingham, UK) were added and the volume made up to 2 l with distilled water. The glucose oxidase solution was stored in a dark bottle at 4°C.

Glucose standards were prepared in triplicate by adding 5 ml glucose oxidase solution to 200 µl of glucose solution (100 µg freeze dried glucose (Analar, VWR, Lutterworth, UK) ml^{-1} distilled water) and glucose blanks were prepared in triplicate by adding 5 ml glucose oxidase solution to 200 µl of distilled water. The standards, samples, glucose blanks and method blanks were incubated in a water bath maintained at 40°C for 15 min and were then removed and allowed to stand at room temperature for 1 h. The absorbance was measured at 505 nm using a Beckman DU600 spectophotometer in a 1 cm^3 cuvette. The absorbance of the glucose blank was read against distilled water, with the method blanks,

glucose standards and samples read against the glucose blanks. Starch content was calculated as follows

$$\text{Starch}_{(\text{g kg}^{-1}\text{ DM})} = 0.4555 \text{ x } (\Delta S - \Delta M) \text{ x } 100W \text{ x } \Delta G \qquad \text{Equation 2.7}$$

Where ΔS = sample absorbance, ΔM = method blank, W = weight of sample (g) and ΔG = glucose standard absorption.

2.9 Water soluble carbohydrates

Water soluble carbohydrates were determined by the method described by MAFF (1986). Exactly 200 mg of sample was accurately weighed into a 250 ml shaking bottle, to which 200 ml distilled water was added. The bottle was capped and shaken for 1 h. The extract was filtered (Whatman No1, Maidstone, UK) into a 250 ml conical flask with the first 5 ml being discarded. Exactly 2 ml filtrate was transferred into a glass test tube kept on ice, to which 10 ml anthrone reagent (760 ml concentrated sulphuric acid (Analar, VWR, Lutterworth, UK), 330 ml distilled water, 1 g anthrone (Sigma, Gillingham, UK) and 1 g Thiourea (Sigma, Gillingham, UK)) was added slowly down the side of the test tube from a burette. The contents were carefully mixed and the tube loosely stoppered and placed in a boiling water bath for 20 min. Tubes were removed and placed on ice to reduce the sample temperature as quickly as possible. Absorption was read at 620 nm using a Beckman DU600 spectophotometer in a 1 cm^3 cuvette. Water soluble carbohydrate content (g kg^{-1} DM) was calculated from a standard curve produced using 2 ml of 0, 0.04, 0.08, 0.12, 0.16 and 0.20 mg ml^{-1} of glucose added to 10 ml anthrone reagent following the above procedure.

2.10 Ether extract

Ether extract was determined by the solvent extraction method of FOSS (1987) using the Soxtec apparatus (FOSS, Warrington, UK). Approximately 2 g dried sample was accurately weighed into a cellulose extraction thimble (Whatman, Maidstone, UK) and plugged with defatted cotton wool. Total fat was extracted by boiling the samples in 25 ml 30-40°C petroleum ether (Analar, VWR, Lutterworth, UK) for 30 min. Samples were then rinsed for 30 min, prior to evaporating off the petroleum ether. Ether extract was determined as

$$\text{Ether extract (g kg}^{-1}\text{ DM)} = \left(\frac{\text{weight of fat (g)}}{\text{weight of sample (g)}}\right) \times 1000 \qquad \text{Equation 2.8}$$

2.11 Volatile fatty acids

The volatile fatty acids, acetic, propionic, n-butyric, iso-butyric, n-valeric and iso-valeric acid were determined by gas chromatography using water extracts of defrosted silage by the method of Zhu *et al* (1996) by the Chemistry Department at the Institute of Grassland and Environmental Research (Aberystwyth, UK).

2.12 Condensed tannins

Condensed tannins were determined using an adaptation of the acid-butanol method of Porter *et al.* (1986) as described by Hagerman (2002). Approximately 2 g of oven dried (40°C) sample was accurately weighed into a 50 ml centrifuge tube, to which 20 ml of 700 ml propanone (GPR, VWR, Lutterworth, UK) l^{-1} distilled water was added. The tube was capped and sonicated at room temperature in a sonic water bath, for 30 min. The tube was centrifuged at 2500 xg at 4°C for 10 min. The exact volume of the supernatant was recorded and stored at 4°C. The pellet was resuspended in 20 ml of 700 ml l^{-1} propanone

and extracted in the same manner as previously described. The supernatants were combined and used for tannin analysis. Exactly 1 ml of combined supernatant was added to approximately 0.2 g polyvinylpyrrolidone (PVPP, Sigma, Gillingham , UK) in a glass test tube. The sample was washed with exactly 5 ml absolute methanol (Analar, VWR, Lutterworth, UK) and spun at 2000 xg for 10 min, the supernatant was discarded and the pellet washed a further 3 times. Exactly 7 ml of acid-butanol reagent (50 ml concentrated hydrochloric acid (Analar, VWR, Lutterworth, UK) and 950 ml butan-1-ol (Analar, VWR, Lutterworth, UK)) was added. Exactly 0.2 ml of ferric ammonium sulphate (0.5 g ferric ammonium sulphate dodeca-hydrate in 25 ml 2 M hydrochloric acid (Analar, VWR, Lutterworth, UK)) was added and the sample mixed on a vortex mixer. The tube was loosely capped and placed in a water bath at 95°C for 1.5 h. the tubes were removed and rapidly cooled on ice, prior to being centrifuged at 2000 xg at 4°C for 10 min. The absorbance of the supernatant was read at 550 nm using a Beckman DU600 spectophotometer. The assay was standardised with 0.1 mg Quebracho tannin. Results were compared to a standard graph of tannic acid (Sigma, Gillingham, UK) solutions (0, 10, 20, 30, 40, 50, 75 and 100 mg ml^{-1}) that had been assayed without addition of PVPP, methanol washing or centrifuging since tannic acid does not bind to PVPP and is soluble in methanol.

2.13 Blood

All plasma samples were analysed for urea (Bayer Diagnostic test kit, T01-1823-56), beta-hydroxy butyrate (Randox Laboratories kit, RB1008), albumin (Randox Laboratories kit, AB361) and total protein (Randox Laboratories kit, TP245) using a Bayer Technicon RA100 autoanalyser (Bayer Plc, Newbury, UK). In addition, bovine plasma samples were analysed for glucose (Bayer Diagnostic test kit, T01-1833-56) and non-esterified fatty acids (Wako Chemicals test kit, 99475409) using a Bayer Technicon RA100 autoanalyser.

3.0 EXPERIMENT 1: EVALUATION OF WHOLE-CROP PEAS AND BEANS AS SILAGES; EFFECT OF CULTIVAR, ADDITIVE TYPE AND WILTING ON THE ENSILING PROFILE AND RESULTANT CHEMICAL COMPOSITION.

3.1 Introduction

The production of high protein silages based on red and white clover has been well documented and reviewed (Wilkins and Jones, 2000). A European wide study was carried out between 1997 and 2001 to investigate low-input animal production, based on forage legumes for silage (LEGSIL). The crops investigated included red and white clover, lucerne, galega and lotus (Halling *et al.*, 2001), but both forage peas and beans were excluded from the project. In a review of home grown proteins for animal feeds (Entec, 1997), it was reported that UK farmers considered peas and beans difficult crops to ensile successfully and made minimal use of them as a protein concentrate. Perceived factors restricting the use of these legumes as silages included their low dry matter and water soluble carbohydrate contents, high levels of anti-nutritive factors, moderate protein content, inconsistent crop yield and a high buffering capacity (McDonald *et al.*, 1991).

Reed (1995) has reported that the presence of condensed tannins in forage legumes also has nutritional implications, resulting in reduced intake and digestibility, when fed to ruminants. However, low levels of condensed tannins might confer protection of protein during ensiling and later on during digestion. Peas and beans are both leguminous crops, enabling the fixation of N into the soil for subsequent crops (Bergersen, 1973). Wilkins and Jones (2000) reported that the use of legumes would be extremely beneficial in organic livestock production, reducing fertiliser requirement and increasing home-grown feed protein. Traditionally, pea silages have been produced from forage variety types. Pea breeders have recently developed semi-leafless, combinable varieties that have an

increased grain yield compared to forage types, and have less resultant straw (Wilkins and Jones, 2000). The straw fraction is usually ploughed back into the land as a green manure (Merry *et al.*, 2001). The potential of these semi-leafless, combinable varieties, of both peas and beans for ensilage has not been examined.

Recent work by Fraser *et al.* (2001) has determined that the optimum growth stage for harvesting forage varieties of peas and beans for ensilage as whole-crops are growth stages 205 (Knott, 1987) and 207 (Knott, 1990) respectively. Field wilting of grass has long been used as a method for increasing the dry matter for either silage or hay production (McDonald *et al.*, 1991), but it is unclear as to whether wilting of whole-crop legumes affects the pattern of fermentation and the resultant chemical composition.

Grass silages can be made successfully both with and without an additive (Haigh *et al.*, 1996). Whole-crop pea and bean silages have been produced successfully with and without additives (Fraser *et al.*, 2001), but it is again unclear as to their effects during ensiling, and which class of additive, i.e. fermentation inhibitors, stimulants, sterilants or nutritive (McDonald *et al.*, 1991), is most suitable for the production of whole-crop pea and bean silages.

The objective of this experiment was to evaluate the use of high and low tannin cultivars of spring sown, semi-leafless, combinable peas and beans, and the effect of wilting and additive type on the pattern of fermentation, chemical composition and nutritional value of the resulting silages.

3.2 Material and Methods

3.2.1 Forage production

3.2.1.1 Crop type

Two varieties of spring sown, semi-leafless combinable peas and beans were selected from the 2001 recommended pulse varieties list (NIAB, 2001). The varieties differed in condensed tannin (CT) content as indicated by flower colour, with white flowers being indicative of a low CT content and purple flowers indicative of high levels of CT (Crofts *et al.*, 1980), but were similar in height, disease resistance and standing ability. The pea cultivars used were Racer (purple flowered; Cebeco Seeds, Netherlands), and Croma (white flowered; Cebeco Seeds, Netherlands). The bean cultivars used were Piccadilly (purple flowered; Blondeau, France) and Avon (white flowered; Cebeco Seeds, Netherlands).

3.2.1.2 Crop establishment

The field used (at Harper Adams University College) was a sandy loam with a pH of 6.7 having phosphorous and potassium indices of 7.3 and 3.7 respectively and did not receive any fertilizer. Prior to drilling, the field site was ploughed, sub-soiled and harrowed. The field site was split into three blocks of 36 m x 24 m, each containing four plots of 18 m x 12 m. The four crops were randomly allocated to a plot within each block. The crops were drilled on the 13th April 2001, using a trial plot seed drill (3 m, pneumatic, Accord, Kverneland Group UK Ltd., St. Helens, UK). The bean varieties, Avon and Piccadilly, were drilled at a seed rate of 311 kg ha^{-1} and 262 kg ha^{-1} respectively with a target establishment rate of 40 plants per square metre, whereas the pea varieties, Racer and Croma, were drilled at 220 kg ha^{-1} and 245 kg ha^{-1} in order to obtain a target of 90 and 70 plants per square metre respectively, in accordance with the breeders recommendations.

The higher target establishment rate used for Racer was due to a lower germination rate and smaller plant size compared to Croma (NIAB, 2001).

In order to deter bird damage, a helium filled balloon attached to a kite was placed in the middle of the experimental plots. On the 16th April 2001, black cotton was strung across the plots to further protect the crop against bird damage. This was removed on 2nd July 2001, prior to harvest.

3.2.1.3 Spray regime

The plots were all sprayed with the pre-emergence contact herbicide, paraquat (Gramoxone, 3 l ha^{-1}), and either the residual herbicide pendimethalin/cyanazone (Bullet, 5 l ha^{-1}) or terbutryn/terbuthylazine (Opaguard, 3.4 l ha^{-1}) for peas and beans respectively. Herbicides were applied using a knapsack sprayer, with a 3 m boom, immediately after the plots had been drilled. Following the discovery of pea and bean weevil (*Sitona lineatus*), an insecticide, Cypermethrin (Cyperkil), was applied at the rate of 0.25 l ha^{-1} on the 11th May and 4th June 2001.

3.2.2 Crop harvest and silage production

The plots were harvested at the growth stages suggested by Fraser *et al* (2001), with the peas harvested on 5th July 2001 (83 days post establishment) at growth stage 205 (flat pod, Knott, 1987), and the beans harvested on 27th July 2001 (104 days post establishment) at growth stage 207 (pod fill, Knott, 1990). All plots were harvested using a Haldrup 1500 plot harvester (J. Haldrup a/s, Løgstør, Denmark; cutter bar mower, no conditioner). Half of each plot was weighed, in order to determine yield, and taken directly off the field and processed, whilst the other half was spread out within the plot to facilitate wilting. Following a field wilt of 24 h, the remaining half of each plot was collected and processed in the same manner. The crops were precision chopped to a length of approximately 3 cm

using a trailed forage harvester (3625, John Deere, Nottingham, UK), after being spread in a line on a concrete pad (Plate 1). The processed material was collected in a silage trailer and tipped onto a silage sheet. A sub-sample of chopped material from each plot was stored frozen at –20°C prior to subsequent analysis.

The chopped material was accurately weighed and split into three piles of 50 kg. One of three additives was applied to each pile, at the rate of 4 l t^{-1} FM, using a pressurised hand sprayer. The treated material was turned and mixed well using garden forks to ensure even application of the additive. The additives used were a fermentation enhancer (bacterial inoculant; Whole-crop legume, Biotal, Cardiff, UK), a fermentation inhibitor (620 ml l^{-1} formalin and 320 ml l^{-1} formic acid; F100, FSL Bells Ltd., Corsham, UK) and a negative control (tap water). From each treatment 12 mini silos (~1.5 kg FM) and 1 maxi silo (~20 kg FM) were produced per plot.

Plate 1 Post harvest processing of the pea crop using a trailed forage harvester

3.2.2.1 Mini silo production

Approximately 1.5 kg FM of treated material was placed into a plastic bag (39 cm x 52 cm, 500 gauge; LBS, England) to create each mini silo. Bags were labelled using laminated raffle tickets and then sealed, after extracting the air using a vacuum line, by twisting the neck and sealing with silage tape. The bags were placed into large boxes and weighed down with bricks. After 0, 12, 24, 48, 96 and 240 h post ensiling, two bags from each treatment per plot were taken and stored at –20°C to stop fermentation prior to subsequent analysis.

3.2.2.2 Maxi silo production

Pre-weighed, plastic lined, maxi silos (30 cm diameter x 50 cm length of drainage pipe attached to a wooden base) were filled with approximately 20 kg FM of fresh treated material and consolidated well. The plastic liner was twisted, and sealed using silage tape, and the full weight recorded. Approximately 5 kg of sand was placed on the top of each silo. The silos were stored outside, undercover, prior to opening. Maxi silos were weighed and opened after 120 d, the contents well mixed by hand, and two sub-samples (~1 kg for proximate analysis and 200 g for gas production) were taken and stored frozen at –20°C prior to subsequent analysis.

3.2.3 Analysis

3.2.3.1 Initial crop

Samples of the initial processed, untreated crops were defrosted slowly at +4°C and analysed for dry matter (DM), ash, crude protein (CP), neutral detergent fibre (NDF), water soluble carbohydrates (WSC), starch and condensed tannins by the methods described in Chapter 2.

3.2.3.2 Ensiling profile - time point mini silos

Mini silos were defrosted slowly at +4°C and when completely defrosted, each silo was opened and the contents mixed well by hand. Sub-samples were taken and analysed for DM, pH, CP and ammonia nitrogen (NH_3-N) by the methods described in Chapter 2.

3.2.3.3 Final 120 d silage – maxi silos

The 1 kg sub-samples of final silage from the maxi silos were defrosted slowly at +4°C and analysed for DM, ash, CP, NH_3-N, pH, NDF, neutral cellulase digestibility (NCD), starch, WSC and ether extract (EE) by the methods described in Chapter 2. Since no predictive equation for whole-crop pea and bean silages is available, the metabolisable energy (ME) was calculated using the dried Lucerne equation of Givens (1989), where

$$ME\ (MJ\ kg^{-1}\ DM) = -0.61 + 0.0151[NCD] \qquad \text{Equation 3.1}$$

The 200 g sub-samples were used to determine the rumen fermentation kinetics (*in vitro*) by an adaptation of the method of Theodorou *et al.* (1994), as described in Section 3.2.3.4.

3.2.3.4 In vitro rumen fermentation kinetics

Four mature, ruminally fistulated, Suffolk cross wethers, mean live weight of 90 kg (s.d.=2.1), were fed a basal diet formulated to 1.05 x maintenance requirements (AFRC, 1993). The sheep were housed individually in slatted floor pens, with *ad libitum* access to clean drinking water. The basal diet consisted of good quality field hay and concentrate fed in the ratio of 7:3 on a DM basis, fed in two equal meals per day of 500 g hay and 210 g concentrate at 08:00 and 20:00 h. The concentrate composition (g kg^{-1} FM) as fed, was 485 barley, 220 sugar-beet pulp, 110 hi-pro soya-bean meal, 35 molasses and 150 rapeseed meal.

Rumen fluid was collected from each sheep after 32, 36 and 40 days post dietary adaptation, into pre-warmed vacuum flasks, 4 h post morning feed. The rumen fluid was pooled and strained through four layers of muslin into a warmed (39°C) conical flask that was continually flushed with gaseous CO_2. Exactly 2.25 l of strained, non blended, rumen fluid was added to 12.75 l of media (Huntington *et al.*, 1998) to create a 150 ml l^{-1} rumen fluid solution and this was kept in a water bath at 39°C under a stream of gaseous CO_2. Media consisted (g l^{-1} weight per volume de-ionised water) of 0.0132 calcium chloride di-hydrate, 0.1 manganese chloride tetra-hydrate, 0.0151 cobalt chloride hexa-hydrate, iron (III) chloride hexa-hydrate, 0.8 ammonium hydrogen carbonate, 7 sodium hydrogen carbonate, 1.89 di-sodium hydrogen ortho-phosphate dodeca-hydrate, 1.24 potassium di-hydrogen ortho-phosphate, 0.12 magnesium sulphate hepta-hydrate, cysteine hydrochloide mono-hydrate and 0.02 resazurin. All reagents were analytical grade. Media was made the day prior to rumen fluid collection and was stored at +4°C. On the day of rumen fluid collection the media was autoclaved (held at 120°C for 20 min) and cooled under a stream of gaseous CO_2 before being placed in an incubator maintained at 39°C.

Approximately 5 g of defrosted final silage from the maxi silos was accurately weighed into a 250 ml Duran bottle and stored at +4°C. Exactly 200 ml of rumen fluid solution was

dispensed into each bottle using a peristaltic pump under a stream of gaseous CO_2 and the bottle was sealed using a modified cap (Plate 2). Three blanks that contained no forage were also dispensed and sealed.

The inside lip of the Schott (GL 45) cap was removed using a lathe and a 12.7 mm hole drilled through the centre of the cap. A stainless steel washer (12.7 mm internal diameter, 41 mm outside diameter, 2 mm depth; Stainless Steel Fasteners Ltd., Chesterfield, UK) was secured using silicone sealant to the underside of the cap. A butyl rubber bung (12.7 to 13 mm tapered; Belko Glass, USA) was pushed through the cap and the washer, and a butyl 'O' ring (40 mm diameter; R.S. Components, Bristol, UK) was placed below the washer (Plate 3).

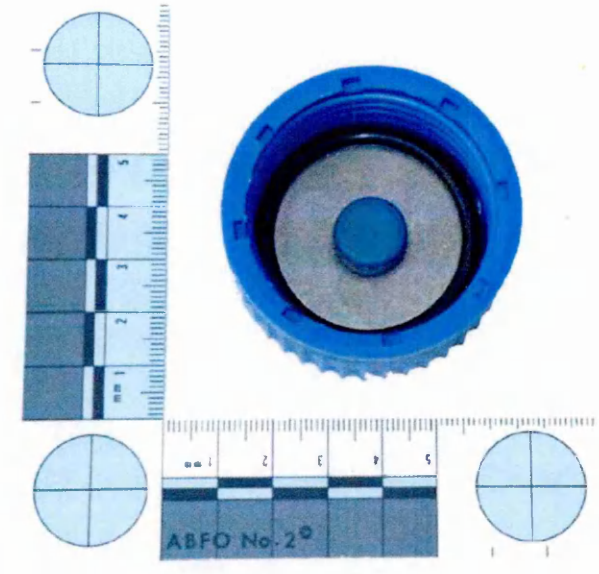


Plate 2 Modified Schott cap used for gas production

Plate 3 Components of the modified Schott cap used for gas production, (from left to right) normal Schott cap, centre drilled Schott cap with lip removed, stainless steel washer, butyl O ring and butyl rubber bung.

During sealing, a 25.4 mm x 18 gauge needle was pushed through the butyl stopper to equalise the pressure. Once all of the bottles were sealed, the needles were removed and the bottles incubated in an incubator maintained at 39°C. Gas pressure (pounds per square inch; psi) within the bottles was recorded at 2, 4, 6, 8, 10, 12, 18, 22, 26, 30, 34, 42, 50, 58, 66 and 72 h post incubation by placing a syringe needle (25.4 mm x 18 gauge), connected to a pressure transducer (T443; Bailey and MacKay Ltd., Birmingham UK), through the butyl bung. The pressure transducer was connected to an IBM compatible personal computer and the data imported directly into a Microsoft Excel spreadsheet using Software Wedge (version 1.1b; T.A.L. Enterprises; Philadelphia, USA) as described by Mauricio *et al.* (1999). After sampling, the needles were withdrawn, the bottles swirled three times, and placed back into the incubator.

After the 72 h sampling, the lids were removed and the bottle contents filtered under vacuum through a dried, pre-weighed sintered glass crucible (50 ml, porosity P1, Pyrex). The crucibles were dried overnight at 100°C, cooled in a desiccator and reweighed. The crucibles were then heated to, and maintained at, 500°C for 12 h in a muffle furnace, removed and allowed to cool in a desiccator prior to being weighed in order to determine the residual ash. The *in vitro* dry matter (IVDMD) and *in vitro* organic matter degradability (IVDOMD) were calculated, after correction for the blank, as follows

$$\text{In vitro digestibility of } X = \frac{(X_{in} - X_{out})}{X_{in}} \qquad \text{Equation 3.2}$$

Where X is either DM or OM.

Gas production (G_p; ml) was predicted from pressure transducer readings (P_t; psi) using the relationship stated in Boyle's Gas Law.

$$G_p = \frac{V_h}{P_a} \times P_t \qquad \text{Equation 3.3}$$

Where V_h represents headspace volume (ml) and P_a is an average standard atmospheric pressure (14.7 psi; Metrological Office, Bracknell, UK). A mean V_h of 107.55 ml (s.d.=4.41) was determined by filling 100 250 ml Duran bottles to the brim with water, recording the exact volume and subtracting 200 (i.e. volume of media). From this, a standard predictive equation was derived, where $G_p=7.32P_t$. After correction for the substrate blanks, cumulative gas production was calculated for 1 g of DM. Gas production profiles were fitted to the model of France *et al.* (1993) using a Genstat program (Dhanoa, *Personal communication*), where

$$G = A - B \times Q^t \times Z^{\sqrt{t}} \qquad \text{Equation 3.4}$$

and was transformed to the functional form (as described by France *et al.* (1993))

$$Y = A\{1 - \exp[-b(t-T) - c(\sqrt{t} - \sqrt{T})]\} \qquad \text{Equation 3.5}$$

Where Y denotes cumulative gas production (ml, calculated using Equation 3.3), t the incubation time (h), A the asymptote (ml), b the underlying rate, c the time dependant rate and T is a discrete lag (h). From this model, a fractional rate of fermentation μ (h^{-1}) was calculated according to Equation 3.6, assuming that the conditions $t \geq T$, $b > 0$ and $c \geq -2b\sqrt{T}$ were satisfied.

$$\mu = b + \frac{c}{2\sqrt{t}} \qquad \text{Equation 3.6}$$

3.2.3.5 Statistical analysis

All measured parameters were analysed using the ANOVA procedure of Genstat (version 6: VSN International, Oxford, UK). Crop yields were analysed in a 2 x 2 (crop x tannin) factorial design using 'field blocks' as a blocking factor. The data from the ensiling profile and the final silage was blocked by 'field block' and analysed using a 2 x 2 x 2 x 3

(Legume x Tannin x Wilt x Additive) factorial design. The same treatment structure was used for the analysis of the fermentation kinetics, but the blocking factor used was run number. Data from the first gas production run was omitted from analysis due to a computer error. This resulted in 6 replicates per treatment.

3.3 Results

3.3.1 Initial crop

Crop yields (t ha^{-1}) are shown in Table 3.1. Beans yielded an additional 8.5 t FM ha^{-1} compared to peas, but there was no difference between peas and beans in DM yield, with mean values of 5.97 vs. 6.07 t DM ha^{-1} respectively. Peas yielded an additional 171 kg CP ha^{-1} compared to the beans. There was also no significant effect of tannin or the interaction between crop and tannin on FM, DM or CP yield.

Table 3.1 Fresh matter (FM), dry matter (DM) and crude protein (CP) yields of low tannin (LT) and high tannin (HT) peas and beans (t ha^{-1})

					Significance					
	Pea		Bean		Crop		Tannin		Interaction	
	LT	HT	LT	HT	s.e.d.	*P*	s.e.d.	*P*	s.e.d.	*P*
FM	25.6	26.5	35.7	33.5	1.74	0.003	1.74	0.730	2.46	0.403
DM	5.93	6.02	6.09	5.97	0.335	0.871	0.335	0.970	0.473	0.775
CP	0.91	0.98	0.83	0.72	0.047	0.010	0.047	0.616	0.066	0.112

Due to a freezer malfunction several pre-ensiling samples were lost and therefore, statistical analysis was not possible. The chemical composition of the crops pre-ensiling is shown in Table 3.2. Wilting in the field for 24 h increased the mean crop DM content from 202 to 244 g kg^{-1} FM. However, the 0 h wilted peas had a higher DM than the 24 h wilted beans (229 and 205 g kg^{-1} FM respectively). The OM and starch content of all the crops were similar, with mean values of 932 and 80 g kg^{-1} DM respectively. The CP and WSC content was highest in the peas, whereas the NDF content was highest in the beans. The major difference between the cultivars was the level of tannin, with the high tannin variety of peas having 1.8x the level of tannin compared to the low tannin variety, and there was 3.9x the amount of tannin in the high tannin beans compared to the low tannin beans. Overall, the high tannin variety of beans contained more tannin than the high tannin variety of pea.

Table 3.2 Initial chemical composition of fresh and wilted, precision chopped, whole-crop peas and beans varying in tannin content (all g kg^{-1} DM unless otherwise stated)

	Pea				Bean			
	0 h Wilt		24 h Wilt		0 h Wilt		24 h Wilt	
	Low Tannin	High Tannin	Low Tannin	High Tannin	Low Tannin	High Tannin	Low Tannin	High Tannin
DM (g kg^{-1} FM)	232	228	275	289	171	179	202	208
OM	937	944	941	928	927	930	925	927
CP	154	162	155	158	136	120	132	115
NDF	224	222	218	215	295	303	305	295
WSC	79.9	80.5	80.6	80.1	48.0	57.5	56.9	64.8
Starch	92.9	69.0	88.2	53.8	81.0	66.1	102.6	67.8
Tannin (TAE†)	13.6	25.0	17.5	32.0	11.6	52.0	9.6	30.2

† Tannic acid equivalents g kg^{-1} DM

3.3.2 Ensiling profile

3.3.2.1 Effect of tannin level, wilting and additive type on the pH of whole-crop peas and beans during ensiling

The effects of tannin level, wilting and additive type on pH of whole-crop peas and beans during ensiling are presented in Table 3.3. The initial pH of the peas was pH 0.6 higher ($P<0.001$) than that of the beans (pH 5.7 and 5.1 respectively, Table 3.3, Figure 3.1). After 96 h the pH of the beans was stable, whereas the pH of the peas was still falling (Figure 3.1). There was little difference between the high and low tannin cultivars up until 48 h post ensiling, after which the high tannin cultivars had a higher ($P<0.001$) pH (Figure 3.2). Wilting for 24 h resulted in an increased ($P<0.010$) pH at 12, 48, 96 and 240 h post ensiling compared to the unwilted material (Figure 3.3).

The use of the fermentation inhibitor resulted in a rapid initial pH drop to pH 5.0, 0.6 lower than both the enhancer and control treatments (Figure 3.4). The use of the fermentation enhancer and control treatment resulted in a similar pattern of pH reduction. However, the enhancer treatment had a lower ($P<0.050$) pH from 48 h post ensiling compared to the control treatment (Figure 3.4), with an overall change of pH 1.4 between 0 and 240 h post ensiling (Table 3.3). The use of the inhibitor only resulted in a change of pH 0.5 over the same period (Table 3.3) with a slower rate of pH reduction (Figure 3.4). There was no legume x wilt interaction at 0 h post ensiling. However, at all the other time points the 24 h wilted pea had a higher ($P<0.050$) pH than the 0 h wilted pea, whereas the 0 h wilted bean had a higher ($P<0.050$) pH than the 24 h wilted bean at 12 and 24 h post ensiling. At all other time points there was no difference between the pH of 0 h and 24 h wilted beans (Figure 3.5).

There was a legume x additive interaction ($P<0.050$) in pH at all measured time points (Figure 3.6), with the fermentation inhibitor treated peas having a lower pH compared to

the enhancer or control up until 24 h post ensiling from where it was higher ($P<0.050$). The pH of the enhancer and control treated peas did not differ at 0, 12 and 24 h post ensiling but from 48 to 240 h post ensiling the enhancer treated peas had a lower ($P<0.050$) pH. The fermentation inhibitor treated beans also had a lower ($P<0.050$) pH up until 24 h post ensiling. At 24 h post ensiling, there was no difference between the inhibitor and enhancer treatments (pH 4.7 and 4.8 respectively, s.e.d.=0.11) or the enhancer and control treatments (pH 4.8 and 4.9 respectively, s.e.d.=0.11). From 48 h post ensiling, the inhibitor treated beans had a higher ($P<0.050$) pH compared to the enhancer or control treated beans. There was no difference in pH between enhancer and control treated beans from 48 to 240 h post ensiling. There was an additive x wilt interaction at 24 h post ensiling, with the control treatment having the highest ($P<0.050$) pH in the 0 h wilted crops whereas the pH was highest in the inhibitor treated crops when wilted for 24 h (Table 3.3).

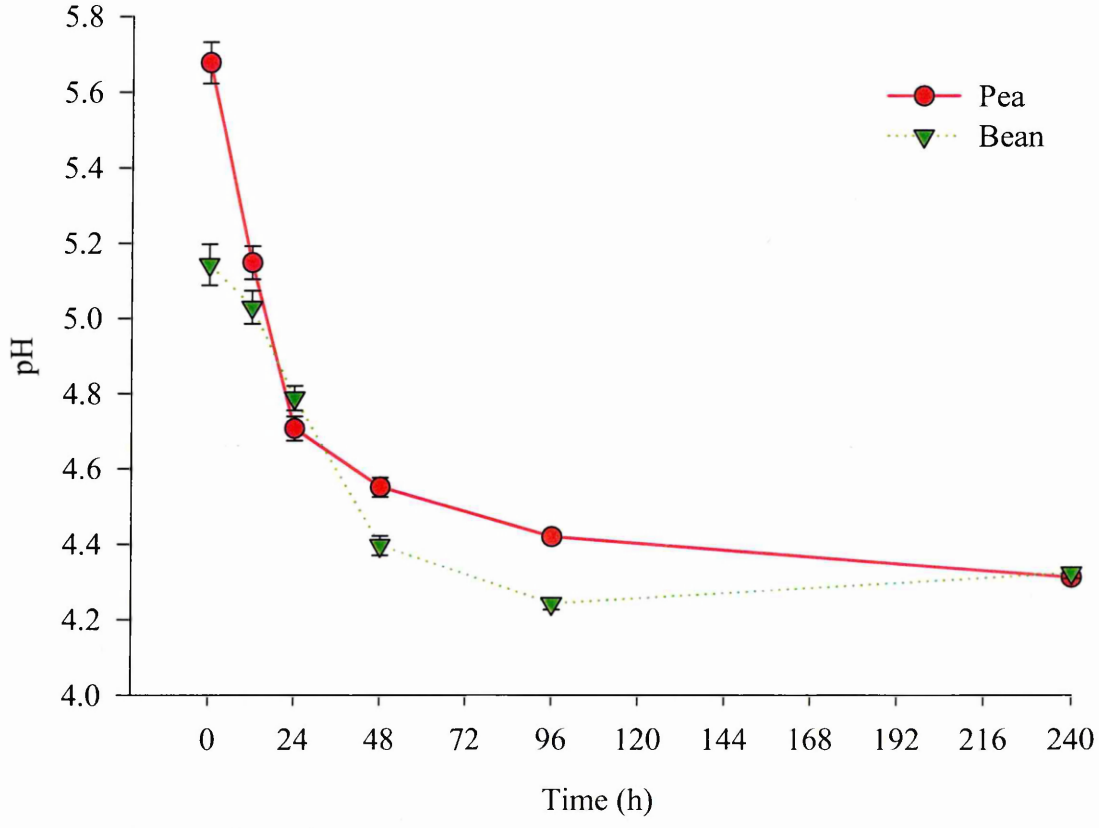


Figure 3.1 Main effect of legume type on pH during ensiling of whole-crop peas and beans during ensiling

Table 3.3 Effect of tannin level, wilting and additive type on the pH of whole-crop peas and beans during ensiling

Legume	Tannin	Wilt	Additive	Time post ensiling (h)					
				0	12	24	48	96	240
Pea	Low tannin	0 h	Inhibitor	5.0	5.0	4.8	4.7	4.5	4.3
			Enhancer	5.9	4.8	4.4	4.3	4.2	4.1
			Control	5.9	4.7	4.5	4.4	4.2	4.1
		24 h	Inhibitor	5.1	5.0	5.0	4.9	4.6	4.6
			Enhancer	6.0	5.6	4.8	4.5	4.3	4.2
			Control	6.0	5.6	4.7	4.6	4.5	4.3
	High tannin	0 h	Inhibitor	5.3	4.9	4.8	4.6	4.5	4.3
			Enhancer	5.9	4.7	4.4	4.3	4.2	4.1
			Control	5.9	4.8	4.5	4.4	4.3	4.2
		24 h	Inhibitor	5.3	5.2	4.9	4.9	4.8	4.7
			Enhancer	5.9	5.7	4.8	4.5	4.5	4.3
			Control	5.9	5.6	4.9	4.7	4.5	4.4
Bean	Low tannin	0 h	Inhibitor	4.6	4.6	4.7	4.4	4.4	4.4
			Enhancer	5.3	5.3	4.9	4.4	4.1	4.1
			Control	5.3	5.3	5.3	4.4	4.1	4.1
		24 h	Inhibitor	4.7	4.7	4.7	4.6	4.4	4.5
			Enhancer	5.1	5.0	4.5	4.2	4.1	4.1
			Control	5.3	4.8	4.6	4.2	4.1	4.1
	High tannin	0 h	Inhibitor	4.6	4.7	4.7	4.7	4.6	4.5
			Enhancer	5.2	5.6	5.0	4.3	4.1	4.2
			Control	5.4	5.5	5.1	4.5	4.2	4.1
		24 h	Inhibitor	5.4	4.8	4.7	4.5	4.4	4.4
			Enhancer	5.3	5.1	4.6	4.3	4.2	4.1
			Control	5.4	4.9	4.6	4.3	4.2	4.2
Significance			s.e.d.	0.19	0.15	0.11	0.09	0.05	0.05
			P	0.312	0.539	0.709	0.147	0.070	0.209
	Main effects		*P* legume (L)	<0.001	0.008	0.013	<0.001	<0.001	<0.001
			P tannin (T)	0.045	0.082	0.643	0.070	<0.001	<0.001
			P wilt (W)	0.094	<0.001	0.358	0.008	<0.001	<0.001
			P additive (A)	<0.001	<0.001	0.010	<0.001	<0.001	<0.001
			P LxT	0.356	0.222	0.770	0.908	0.944	0.352
			P LxW	0.438	<0.001	<0.001	<0.001	<0.001	<0.001
			P LxA	0.027	0.003	<0.001	0.045	0.050	0.023
			P TxW	0.212	0.938	0.373	0.604	0.652	0.820
			P TxA	0.127	0.990	0.504	0.522	0.926	0.810
			P WxA	0.262	0.553	0.004	0.325	0.419	0.056
			P LxTxW	0.061	0.240	0.966	0.224	0.143	0.006
			P LxTxA	0.990	0.711	0.168	0.459	0.671	0.675
			P LxWxA	0.155	<0.001	<0.001	0.333	0.347	0.004
			P TxWxA	0.499	0.519	0.302	0.294	0.382	0.078

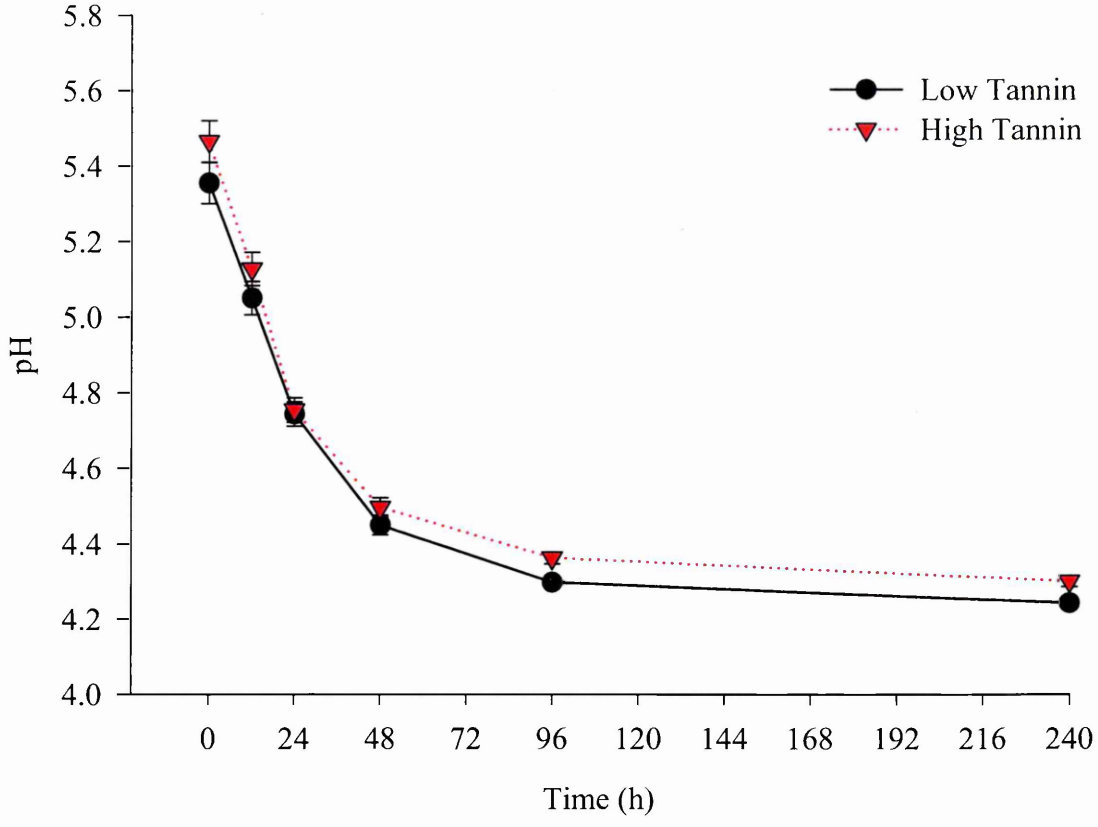


Figure 3.2 Main effect of tannin level on pH during ensiling of whole-crop peas and beans

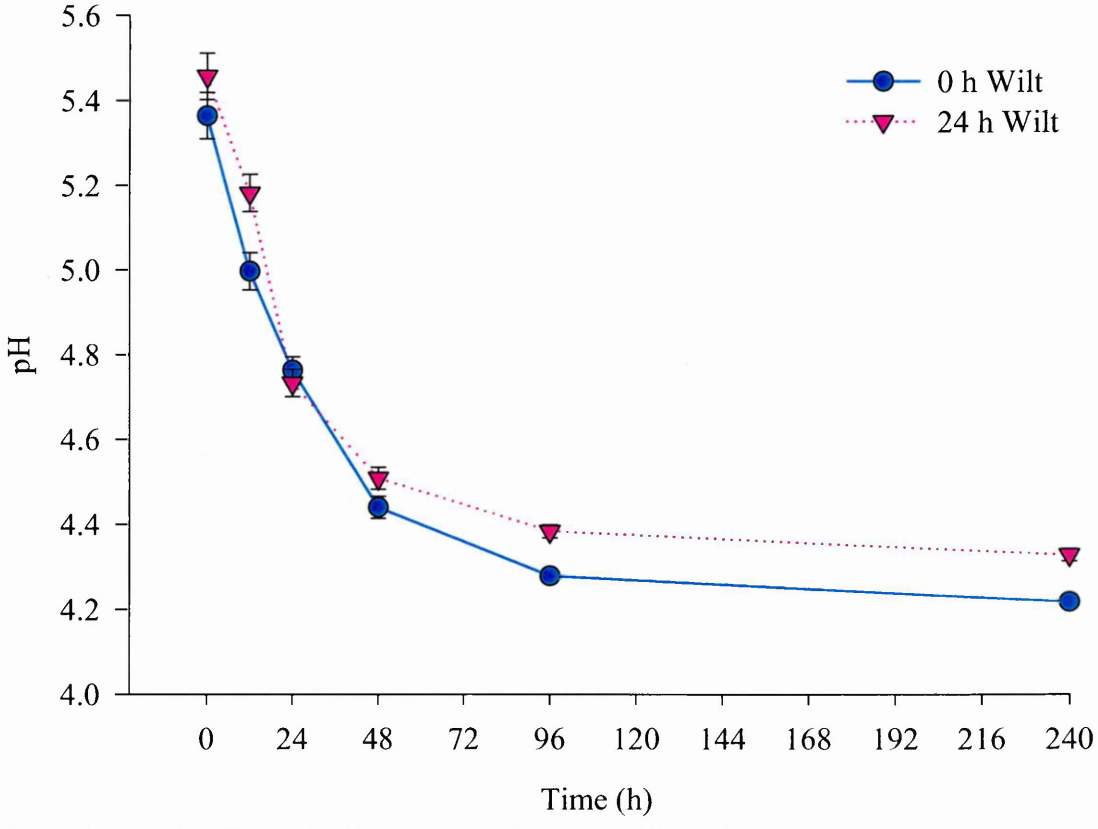


Figure 3.3 Main effect of wilting on pH during ensiling of whole-crop peas and beans

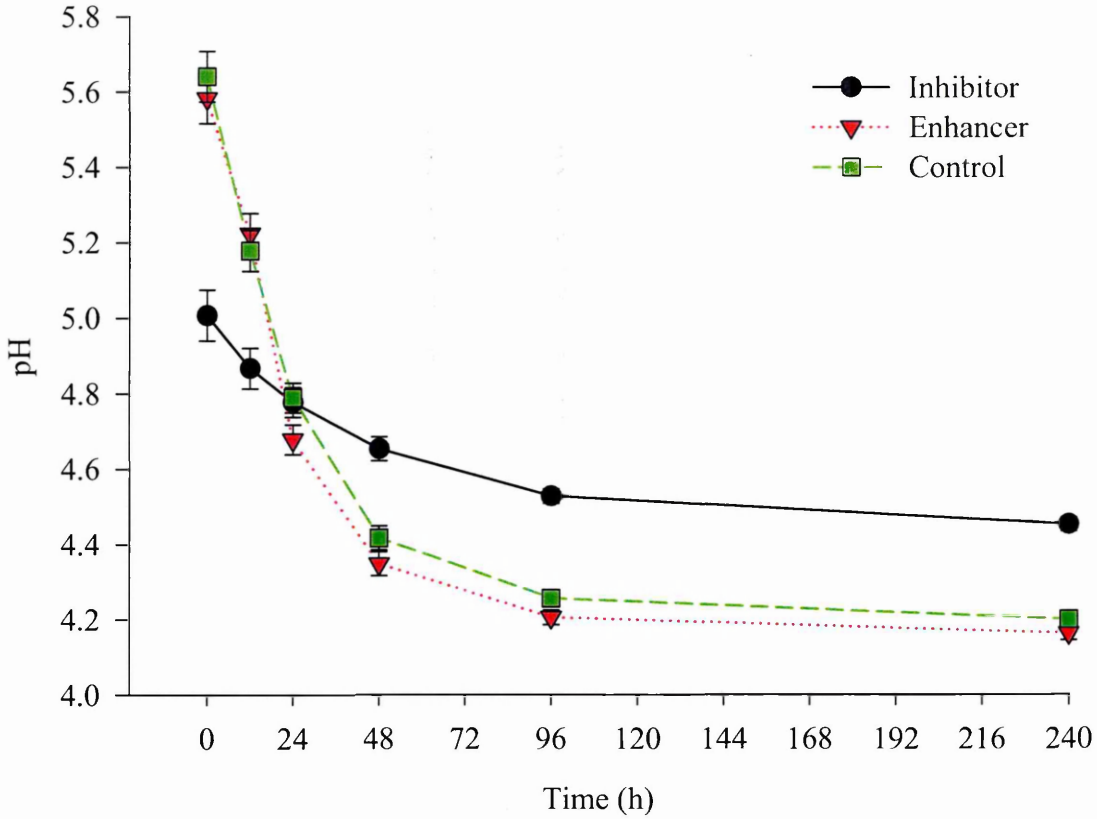


Figure 3.4 Main effect of additive type on pH during ensiling of whole-crop peas and beans

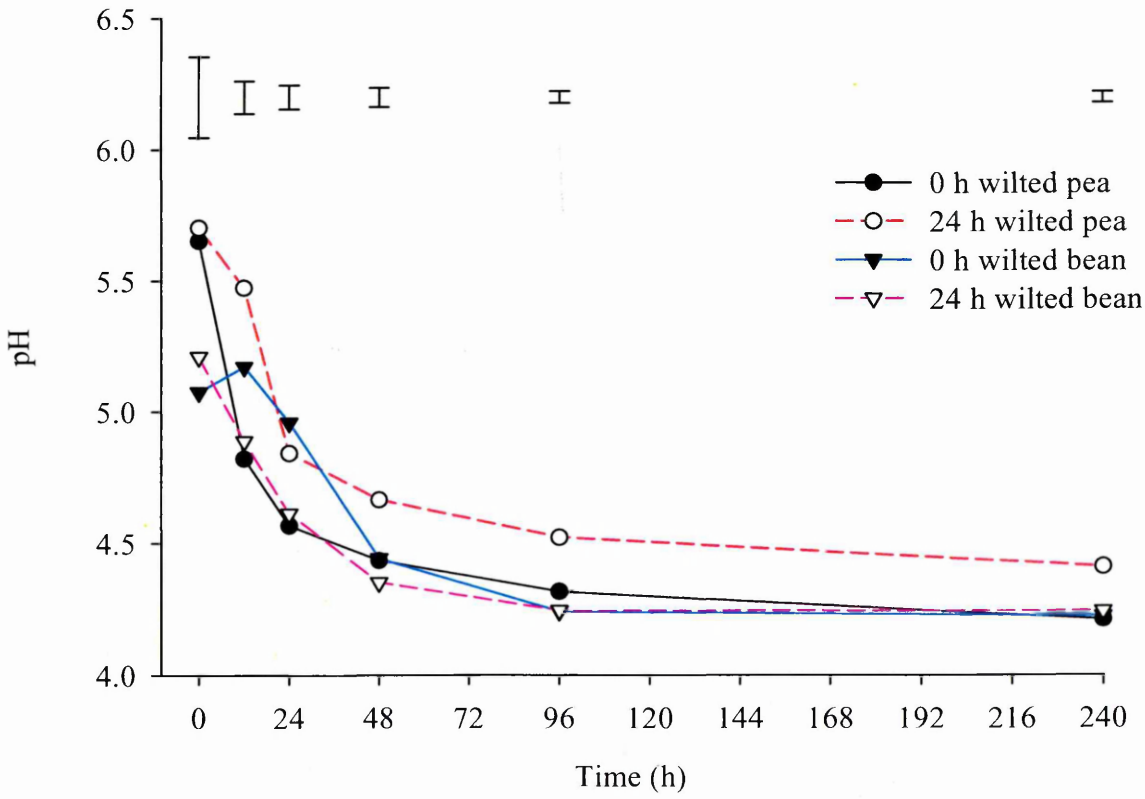


Figure 3.5 Effect of legume type and wilting on pH during ensiling of whole-crop peas and beans

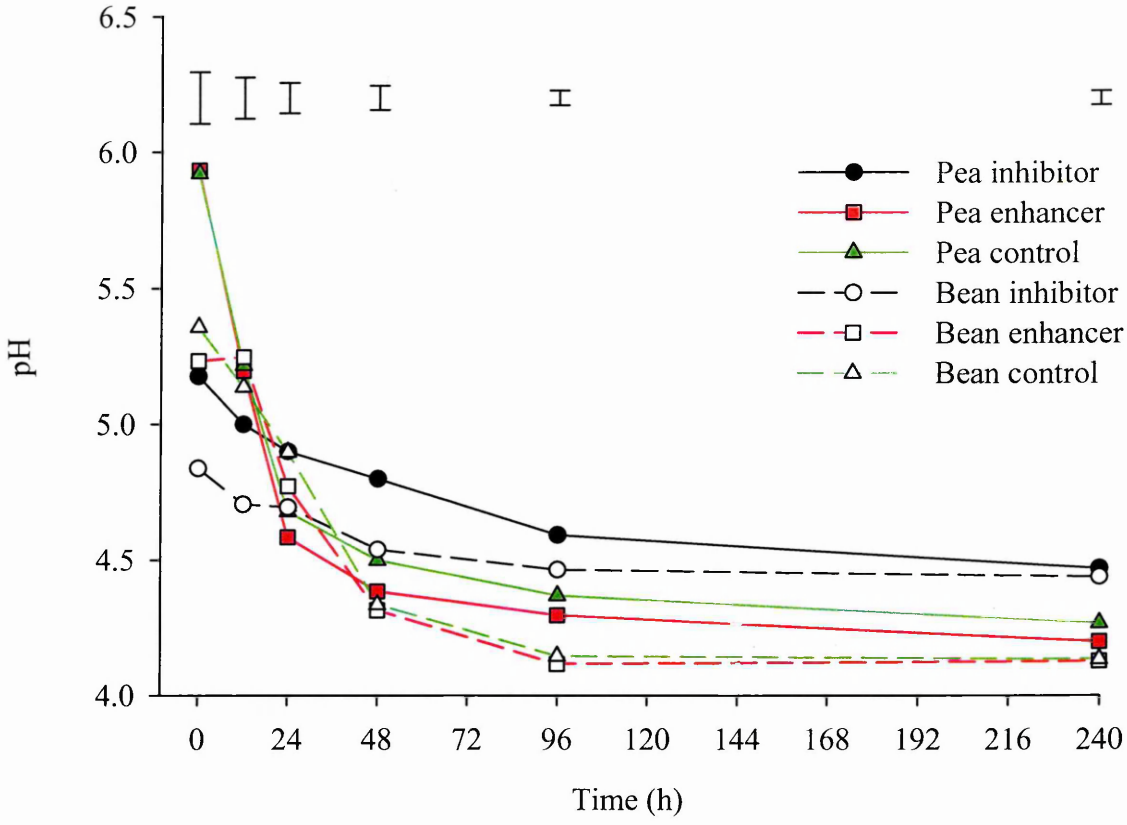


Figure 3.6 Effect of legume type and additive type on pH during ensiling of whole-crop peas and beans

3.3.2.2 Effect of tannin level, additive type and wilting on the ammonia nitrogen content of whole-crop peas and beans during ensiling

The effect of tannin, wilting and additive type on the ammonia nitrogen content of whole-crop peas and beans during ensiling is presented in Table 3.4. Peas initially had a lower (P<0.001) NH_3-N content than beans at 0 h post ensiling. From 96 h post ensiling the rate of proteolysis in the peas had slowed whereas the rate of ammonia formation in beans continued to increase up until 240 h post ensiling (Figure 3.7). High tannin cultivars had a lower (P<0.001) NH_3-N content from 12 h post ensiling compared with the low tannin cultivars (Figure 3.8).

There was little effect of length of wilting on NH_3-N content up until 96 h post ensiling, after which silages wilted for 24 h had a lower (P<0.050) NH_3-N content compared to

those wilted for 0 h (Figure 3.9). The use of the fermentation inhibitor resulted in a lower (P<0.010) NH_3-N content at all measured time points, with the amount increasing linearly over time. The use of the fermentation enhancer or control treatments resulted in similar, curvilinear, concentrations of NH_3-N (Figure 3.10). There was a legume x tannin interaction (P<0.001) at 0 h post ensiling, due to the high tannin pea cultivar having a higher (P<0.050) NH_3-N content compared to the low tannin cultivar of peas, with the opposite in the beans (Table 3.4). The interaction (P<0.050) at 12 and 24 h post ensiling was due to the high tannin pea cultivar having a lower (P<0.050) NH_3-N content than the low tannin pea cultivar with there being no difference between bean cultivars.

The interactions between legume type and wilting are presented graphically in Figure 3.11. The 0 h wilted peas had the highest (P<0.050) NH_3-N content at 12 h post ensiling, with no difference between the other treatments. At 24 h post ensiling the 24 h wilted beans had the lowest NH_3-N content with no difference between the other treatments, whilst at 240 h post ensiling there was no difference between wilting and peas but the 0 h wilted beans had a higher (P<0.050) NH_3-N content compared to the 24 h wilted beans. The legume x additive interaction (P<0.001) at 12 and 24 h post ensiling were caused by the inhibitor treated crops having the lowest pH, with the value for beans being higher (P<0.050) than that for the peas, with no difference between the enhancer and control treated beans at 12 h post ensiling and peas 24 h post ensiling (Table 3.4). The control treated peas had the highest (P<0.050) NH_3-N content at 12 h post ensiling, whereas the control treated beans had a higher (P<0.050) NH_3-N content compared to the enhancer treated beans 24 h post ensiling.

The tannin x additive interactions (P<0.010) at 24 and 48 h post ensiling were caused by the inhibitor treated silages having the lowest (P<0.050) NH_3-N content across both tannin levels, whilst the enhancer and control treated low tannin silages had higher (P<0.050) NH_3-N contents compared to the enhancer and control treated high tannin silages. There

was a wilting x additive interaction (P<0.010) at 0 and 96 h post ensiling; at 0 h post ensiling the 0 h wilted crops treated with the inhibitor had a lower NH_3-N content compared to the other treatments, but there was no difference between NH_3-N content of crops wilted for 24 h. After ensiling for 240 h, the inhibitor treated crops had the lowest (P<0.050) NH_3-N content after both 0 and 24 h wilting with values of 18.7 and 24.2 g kg^{-1} TN (s.e.d.=2.30) respectively. The mean NH_3-N content of the enhancer and control treatments was 47.2 and 41.1 g kg^{-1} TN for 0 and 24 h wilt respectively.

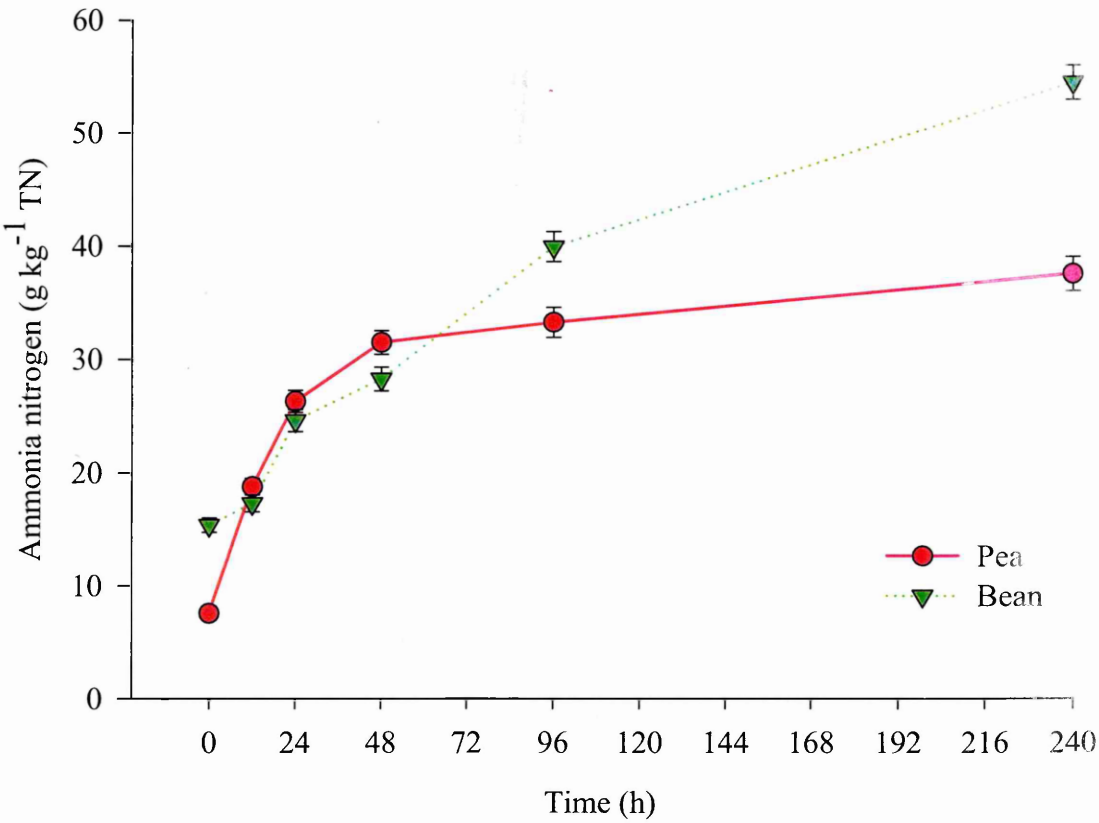


Figure 3.7 Main effect of legume type on change in NH_3-N content during ensiling of whole-crop peas and beans

Table 3.4 Effect of tannin level, wilting and additive type on the ammonia nitrogen content of whole-crop peas and beans during ensiling (all g kg^{-1} TN)

Legume	Tannin	Wilt	Additive	Time post ensiling (h) 0	12	24	48	96	240
Pea	Low tannin	0 h	Inhibitor	5.2	9.2	8.7	16.2	13.5	3.07
			Enhancer	8.6	27.9	39.1	47.3	48.3	47.3
			Control	8.0	34.9	39.8	43.9	44.6	47.7
		24 h	Inhibitor	4.4	7.0	11.0	17.1	26.0	27.2
			Enhancer	6.2	18.2	37.7	45.5	42.9	44.7
			Control	6.8	21.6	39.0	38.0	46.6	51.8
	High tannin	0 h	Inhibitor	5.3	11.6	13.3	16.3	22.4	24.0
			Enhancer	9.2	23.1	30.2	36.1	37.8	38.6
			Control	12.2	27.0	29.4	31.6	35.2	37.0
		24 h	Inhibitor	8.1	7.7	11.9	19.1	18.5	17.1
			Enhancer	8.6	17.1	26.4	33.7	29.0	39.8
			Control	8.1	15.8	29.1	34.0	34.2	44.5
Bean	Low tannin	0 h	Inhibitor	13.6	15.3	18.6	13.6	24.6	40.6
			Enhancer	22.7	20.3	26.2	34.0	52.3	69.4
			Control	20.0	18.4	27.7	37.2	58.0	75.8
		24 h	Inhibitor	16.9	13.5	16.3	17.7	29.1	42.2
			Enhancer	16.8	24.2	32.4	35.1	46.5	57.4
			Control	12.7	22.1	32.0	35.7	47.1	60.6
	High tannin	0 h	Inhibitor	13.0	11.9	13.7	17.1	14.2	32.1
			Enhancer	17.0	16.3	21.2	35.5	46.0	58.8
			Control	17.0	17.0	28.9	31.2	55.4	71.5
		24 h	Inhibitor	9.8	10.6	11.7	17.0	23.1	42.7
			Enhancer	13.3	16.0	26.9	34.6	44.1	50.8
			Control	11.9	18.0	32.7	36.4	38.7	51.8
Significance			s.e.d.	2.19	2.54	3.35	3.59	4.60	5.26
			P	0.043	0.323	0.415	0.757	0.194	0.392
	Main effects		*P* legume (L)	<0.001	0.047	0.083	0.003	<0.001	<0.001
			P tannin (T)	0.262	<0.001	<0.001	<0.001	<0.001	<0.001
			P wilt (W)	<0.001	<0.001	0.139	0.796	0.098	0.020
			P additive (A)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
			P LxT	<0.001	0.397	0.045	0.014	0.595	0.578
			P LxW	0.046	<0.001	0.017	0.582	0.280	0.021
			P LxA	0.199	<0.001	<0.001	0.174	0.088	0.396
			P TxW	0.923	0.554	0.931	0.798	0.208	0.510
			P TxA	0.443	0.193	0.002	0.003	0.291	0.892
			P WxA	0.006	0.510	0.925	0.323	<0.001	0.175
			P LxTxW	0.570	0.227	0.305	0.261	0.114	0.800
			P LxTxA	0.918	0.157	0.004	0.043	0.024	0.701
			P LxWxA	0.687	0.004	0.277	0.863	0.012	<0.001
			P TxWxA	0.533	0.753	0.793	0.390	0.606	0.821

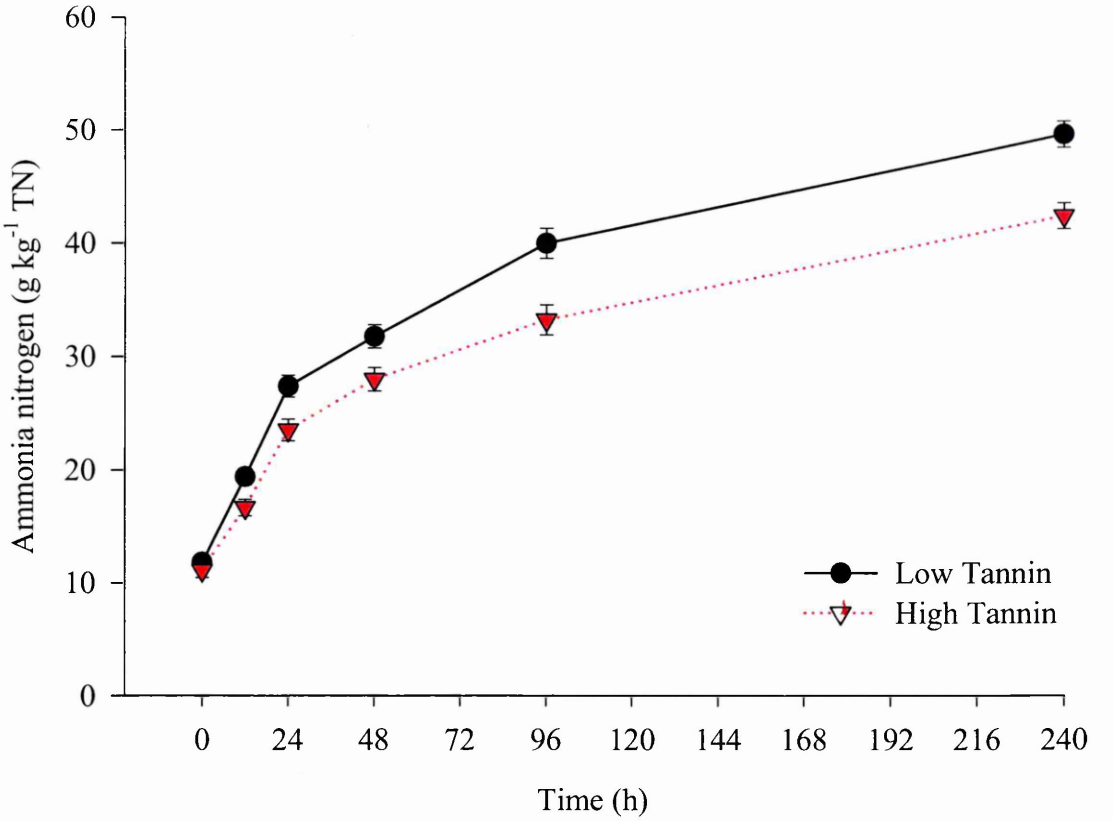


Figure 3.8 Main effect of tannin level on change in NH_3-N content during ensiling of whole-crop peas and beans

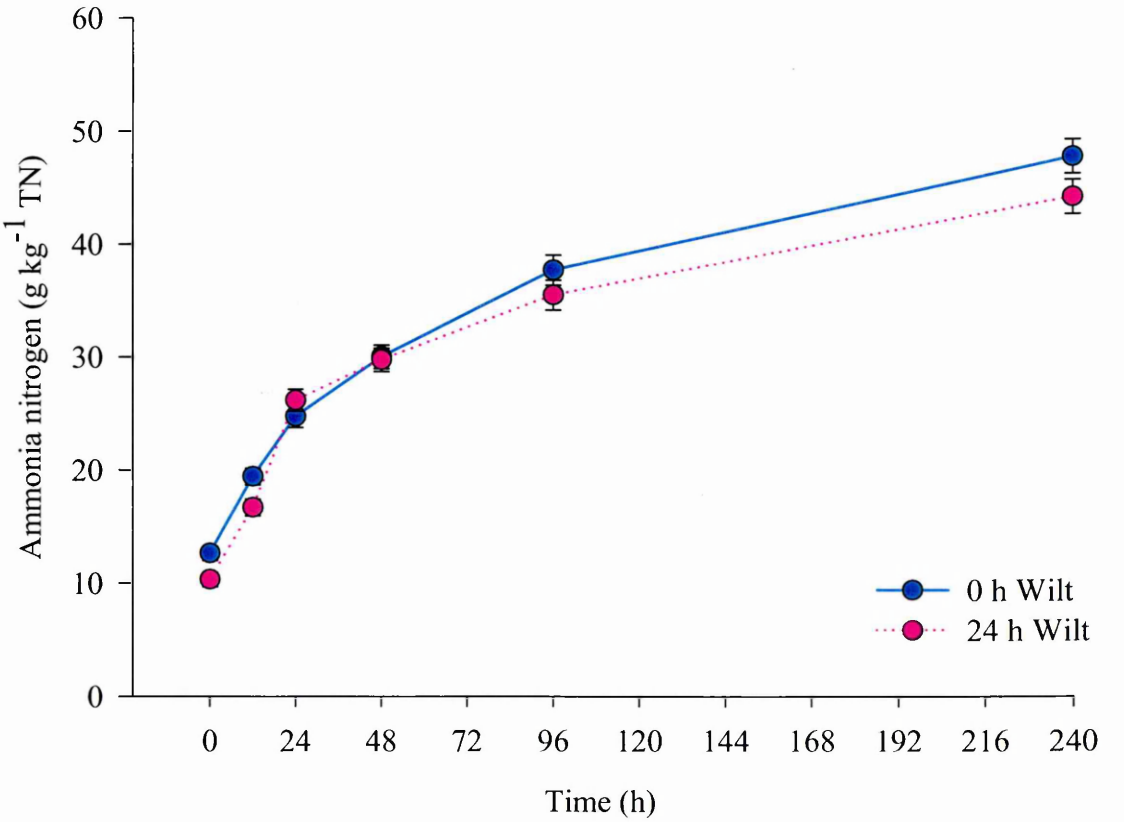


Figure 3.9 Main effect of wilting on the change in NH_3-N content during ensiling of whole-crop peas and beans

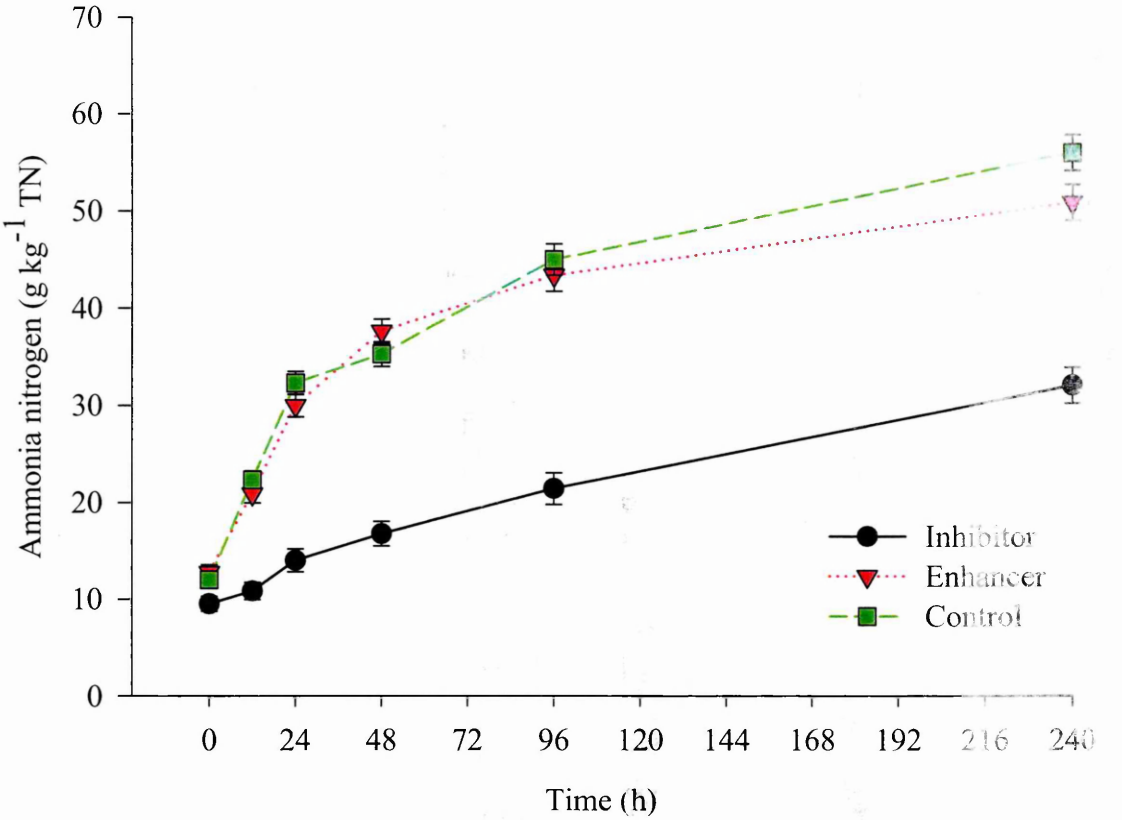


Figure 3.10 Main effect of additive type on the change in NH_3-N content during ensiling of whole-crop peas and beans

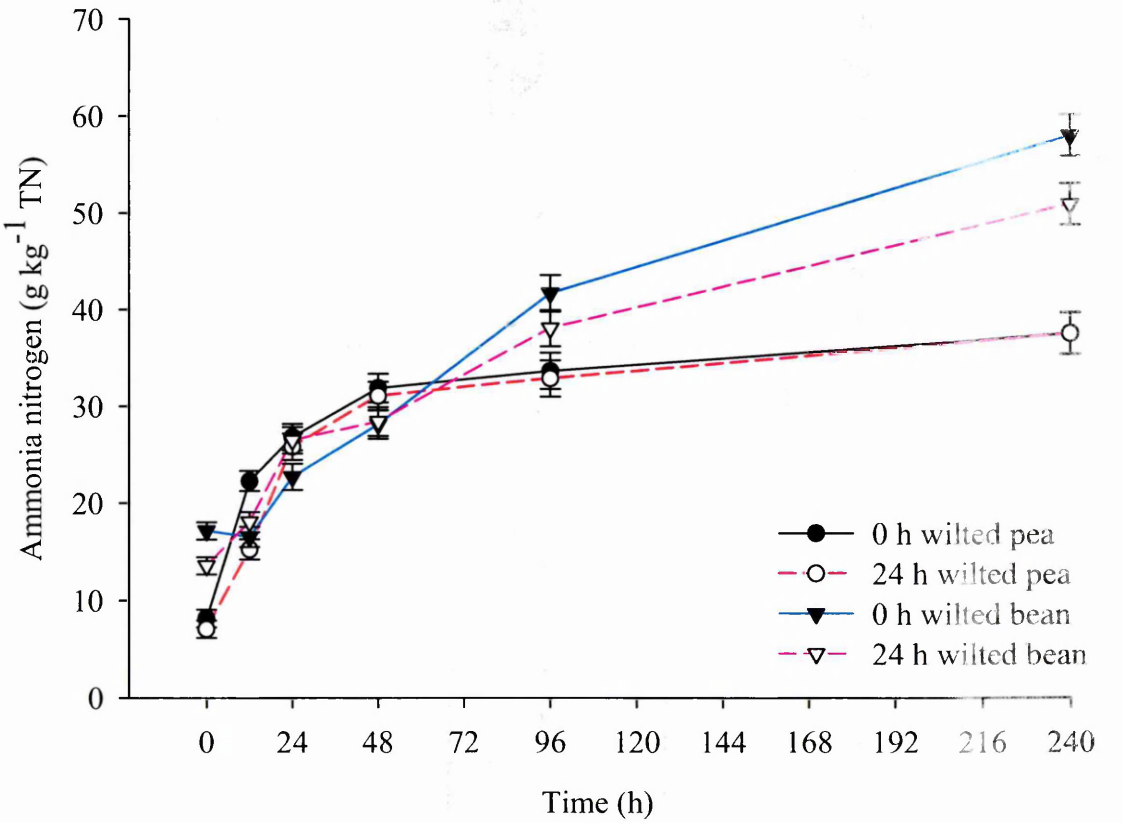


Figure 3.11 Effect of wilting and legume type on change in NH_3-N content during ensiling of whole-crop peas and beans

3.3.2.3 Effect of tannin level, additive type and wilting on the losses of DM and CP of whole-crop peas and beans during ensiling

The DM lost from the pea silages during 12 to 96 h post ensiling was greater than that from the beans (Figure 3.12). However, at 240 h post ensiling the loss of DM was greater (P=0.001) in the bean silages compared to the pea silages, with values of 0.117 and 0.089 kg kg^{-1} respectively (Figure 3.12, s.e.d.= 0.0085).

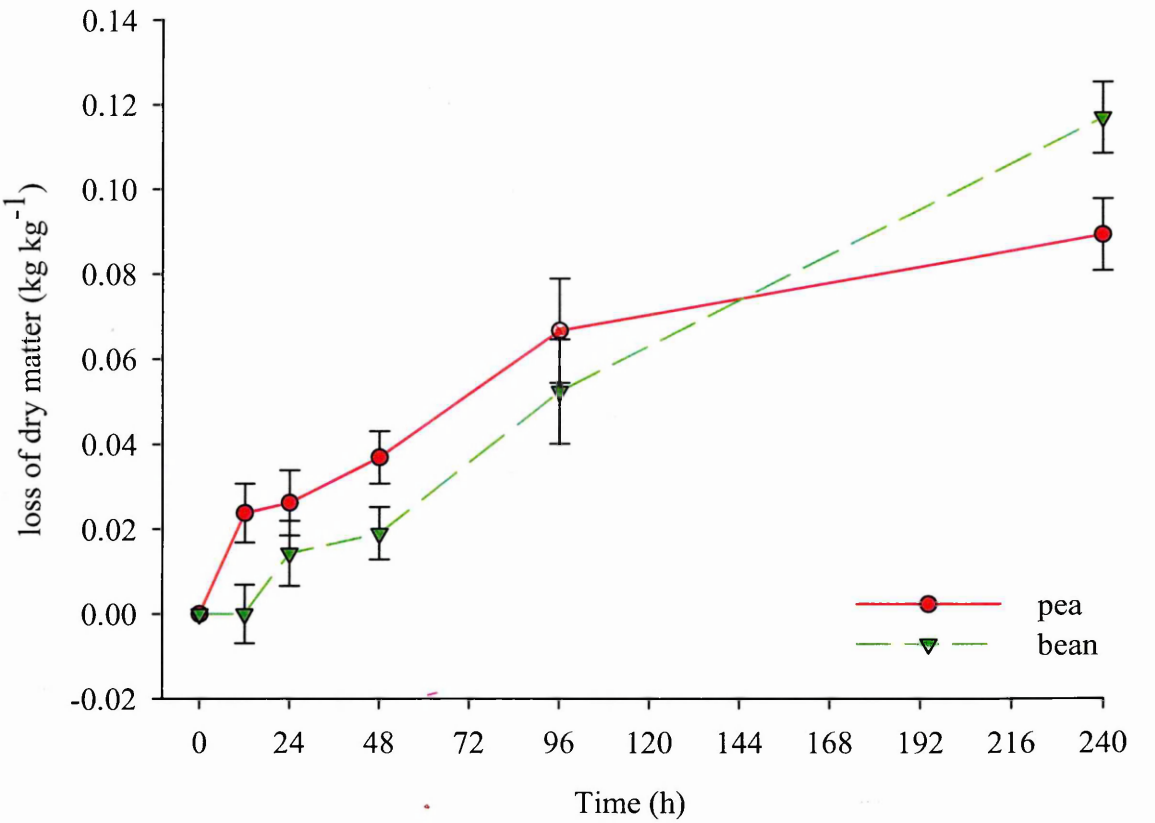


Figure 3.12 Main effect of legume type on dry matter loss during ensiling of whole-crop peas and beans

Dry matter losses were unaffected by tannin level, wilting or additive type during the first 96 h post ensiling. At 240 h post ensiling the effect of wilting peas for 24 h was to reduce (P<0.050) DM loss by 0.046 kg kg^{-1}; there was no effect of wilting on DM loss in bean silages. The high tannin cultivars of the peas and beans led to DM losses of 0.024 and 0.036 kg kg^{-1} (P<0.050) respectively, compared to the low tannin cultivars at 240 h post

ensiling. Additionally, at 240 h post ensiling, the inhibitor treated bean silages had a greater (P<0.050) DM loss of 0.154 kg kg^{-1} compared to 0.090 and 0.106 kg kg^{-1} (s.e.d.=0.0147) for the enhancer and control treated beans respectively, whereas the enhancer treated peas had the highest DM loss of 0.106 kg kg^{-1} compared to 0.087 and 0.075 kg kg^{-1} for the inhibitor and control treated peas respectively.

There was no effect of any treatment on CP loss during the monitored ensiling period, with mean losses of -0.002, -0.010, -0.033, -0.020 and -0.020 kg kg^{-1} at 12, 24, 48, 96 and 240 h post ensiling respectively.

3.3.3 Final 120 d silage

3.3.3.1 Effect of tannin level, additive type and wilting on the ensiling characteristics of whole-crop peas and beans after 120 d ensiling

The final pH of the silages was unaffected by legume type or wilting, with a mean value of pH 4.1 for both treatments (Table 3.5). There was an effect of tannin, with the high tannin cultivars having an increased (P=0.050) pH of 4.2 compared to pH 4.1 for the low tannin cultivars (s.e.d.=0.03). The use of the fermentation inhibitor resulted in a higher (P<0.050) pH compared to the enhancer and control treatments with mean values of pH 4.2, 4.1 and 4.1 respectively (s.e.d.=0.04). There was a wilt x tannin interaction (P=0.014) with the high tannin cultivars having a higher pH than the low tannin cultivars when wilted for 0 h, with no difference being observed between tannin levels when wilted for 24 h.

There was no effect of either legume type or wilting on the NH_3-N content of the final silages. The use of high tannin cultivars led to a reduction (P=0.001) in NH_3-N content with a value of 74.0 g kg^{-1} TN compared to a value of 84.5 g kg^{-1} TN for the low tannin cultivars (s.e.d.=3.04). There was no difference between NH_3-N content of either the enhancer or control treated silages with a mean value of 83.1 g kg^{-1} TN. However, the inhibitor treated silages had a lower (P<0.050) NH_3-N content of 71.5 g kg^{-1} TN (s.e.d.=3.72). Wilting of the pea silages for 24 h increased the NH_3-N content by 7.7 g kg^{-1} TN. In contrast, wilting the bean silages for 24 h reduced the NH_3-N content by 12.3 g kg^{-1} TN. Residual WSC content was affected by legume type, with the pea silages having a greater (P<0.001) WSC content of 22.7 g kg^{-1} DM compared to that of 11.2 g kg^{-1} DM for the bean silages. Neither wilting, tannin level or additive type affected residual WSC content. There was a legume x wilt interaction (P=0.005), with wilting the pea silages for 24 h increasing (P<0.050) the residual WSC, compared to no wilt, but there was no effect of wilting on residual WSC content of the bean silages.

Table 3.5 Effect of tannin level, wilting and additive type on the ensiling characteristics of whole-crop pea and bean silages after 120 d ensiling

Legume	Tannin	Wilt	Additive	Parameter		
				pH	NH_3-N (g kg^{-1} TN)	WSC (g kg^{-1} DM)
Pea	Low tannin	0 h	Inhibitor	4.1	71.0	17.6
			Enhancer	4.0	83.5	20.1
			Control	4.0	85.0	19.4
		24 h	Inhibitor	4.3	81.3	26.6
			Enhancer	4.0	88.2	23.8
			Control	4.0	85.2	23.0
	High tannin	0 h	Inhibitor	4.2	51.8	23.4
			Enhancer	4.2	75.9	23.9
			Control	4.2	70.1	20.9
		24 h	Inhibitor	4.2	72.3	25.4
			Enhancer	4.1	79.0	26.9
			Control	4.1	78.6	21.4
Bean	Low tannin	0 h	Inhibitor	4.2	82.8	10.5
			Enhancer	4.0	89.6	10.9
			Control	4.0	106	16.7
		24 h	Inhibitor	4.2	74.3	8.7
			Enhancer	4.1	82.8	10.5
			Control	4.2	84.8	14.4
	High tannin	0 h	Inhibitor	4.4	75.0	10.3
			Enhancer	4.2	82.7	15.6
			Control	4.0	90.7	10.1
		24 h	Inhibitor	4.1	63.6	8.1
			Enhancer	4.1	76.2	8.5
			Control	4.1	71.6	10.6
Significance			s.e.d.	0.11	10.53	3.43
			P	0.700	0.884	0.353
		Main effects	*P* legume (L)	0.468	0.117	<0.001
			P tannin (T)	0.050	0.001	0.801
			P wilt (W)	1.000	0.474	0.475
			P additive (A)	<0.001	0.003	0.616
			P LxT	0.773	0.875	0.105
			P LxW	0.856	0.002	0.005
			P LxA	0.773	0.615	0.065
			P TxW	0.014	0.656	0.205
			P TxA	0.603	0.779	0.113
			P WxA	0.624	0.360	0.736
			P LxTxW	0.586	0.639	0.596
			P LxTxA	0.292	0.850	0.797
			P LxWxA	0.051	0.542	0.587
			P TxWxA	0.547	0.920	0.703

3.3.3.2 Effect of tannin level, additive type and wilting on the chemical composition of whole-crop peas and beans after 120 d ensiling

The mean DM of the final silage was affected by legume type, with peas having a higher (P<0.001) DM compared to beans (236 and 182 g kg^{-1} FM respectively, s.e.d.=3.0). Wilting in the field for 24 h increased (P<0.001) the mean DM from 191 to 226 g kg^{-1} FM (s.e.d.=3.0, Table 3.6). There was a legume x wilt interaction (P<0.001), with wilting in the field for 24 h having a larger effect on the peas, with an increase in DM of 46 g kg^{-1} FM, compared to an increase of 24 g kg^{-1} FM for the beans. Additive, *per se*, had no effect (P<0.050) on mean DM, but there was a legume x additive interaction (P=0.022, Table 3.6). Control treated peas had a higher (P<0.050) DM compared to the inhibitor and enhancer (243, 231 and 234 g kg^{-1} FM respectively, s.e.d.= 4.2), whereas the enhancer treated beans had the highest DM (190 g kg^{-1} FM, P<0.050) compared to the inhibitor or control (177 and 178 g kg^{-1} FM, respectively, s.e.d.= 4.2). There was no difference between the mean DM of the low and high tannin varieties when wilting for 0 h. However, wilting for 24 h increased (P<0.050) the mean DM of the high tannin varieties to a greater extent than the low tannin varieties (233 and 219 g kg^{-1} FM, s.e.d.= 4.2).

The peas had a higher (P<0.001) OM compared to the beans with mean values of 922 and 912 g kg^{-1} DM respectively (s.e.d.=2.2). There was no effect of wilting or additive type on mean OM content. On average, the high tannin cultivars had a lower (P=0.012) OM compared to the low tannin cultivars, with values of 914 and 920 g kg^{-1} DM respectively (s.e.d.=2.2, Table 3.6). There was a legume x tannin interaction (P<0.001), with the low tannin pea having a higher (P<0.050) OM compared to the high tannin pea variety, but there was no difference between bean cultivars.

Mean CP was affected by legume type, with peas having a higher (P<0.001) CP compared to the beans (177 and 138 g kg^{-1} DM respectively, s.e.d.=2.0). There was an interaction

between legume and tannin, with high tannin peas having a higher (P<0.050) CP compared to the low tannin peas (181 and 174 g kg^{-1} DM respectively, s.e.d.=2.9). Conversely the low tannin beans had a higher (P<0.050) CP compared to the high tannin beans (142 and 133 g kg^{-1} DM respectively, s.e.d.=2.9).

Starch content was not affected by legume type or tannin level. However, wilting in the field for 24 h decreased (P<0.050) the level of starch (101 compared to 118 g kg^{-1} DM, s.e.d.= 6.8). The control additive treatment had the lowest (P<0.050) level of starch, followed by the inhibitor and the enhancer (94, 112 and 123 g kg^{-1} DM respectively, s.e.d.=8.3). The starch content of low tannin peas was higher (P<0.050) than that of the high tannin peas (116 and 97 g kg^{-1} DM respectively, s.e.d.= 9.5), but the reverse was true in beans, where the high tannin variety led to the higher (P<0.050) starch content compared to the low tannin variety (134 and 91 g kg^{-1} DM respectively, s.e.d.= 9.5).

Beans had a higher (P<0.001) NDF content compared to peas (347 and 257 g kg^{-1} DM respectively, s.e.d.= 4.0), whilst the inhibitor treated silages had the highest (P<0.001) NDF content followed by the enhancer and finally the control (315, 302 and 290 g kg^{-1} DM respectively, s.e.d.= 4.8). There were no other effects of treatment on NDF content.

Pea silages had higher (P<0.001) EE contents compared to beans (22.8 and 17.5 g kg^{-1} DM respectively, s.e.d.=0.43, Table 3.6). Wilting the silages for 24 h reduced the EE content by 2 g kg^{-1} DM (Table 3.6). However, higher levels of tannin increased (P<0.001) the EE content by 2 g kg^{-1} DM (Table 3.6). There was no effect of additive on EE content. There was no difference between the high and low tannin bean silages in EE content, whereas the high tannin peas had a greater (P<0.050) EE content at 24.2 g kg^{-1} DM compared to 21.3 g kg^{-1} DM for the low tannin peas (s.e.d.=0.61). There was a legume x additive interaction (P=0.041) caused by the enhancer treatment having the highest (P<0.050) EE content in the peas and the lowest (P<0.050) in the bean silages.

Table 3.6 Effect of tannin level, wilting and additive type on the chemical composition of whole-crop pea and bean silages after 120 d ensiling (all g kg^{-1} DM)

Legume	Tannin	Wilt	Additive	Parameter					
				DM	OM	CP	Starch	NDF	EE
Pea	Low tannin	0 h	Inhibitor	213	931	171	168	271	22.7
			Enhancer	213	933	180	117	262	22.9
			Control	218	931	167	88	253	22.7
		24 h	Inhibitor	241	930	178	104	282	18.3
			Enhancer	251	929	173	115	259	21.7
			Control	263	926	173	101	248	19.6
	High tannin	0 h	Inhibitor	208	920	182	112	271	24.8
			Enhancer	211	906	182	114	248	25.5
			Control	214	919	179	90	243	26.0
		24 h	Inhibitor	262	915	188	86	259	22.2
			Enhancer	260	912	185	109	246	24.3
			Control	276	917	173	101	244	22.3
Bean	Low tannin	0 h	Inhibitor	171	912	146	100	345	17.6
			Enhancer	174	919	142	121	351	16.4
			Control	168	908	131	71	332	18.3
		24 h	Inhibitor	182	898	145	72	360	16.2
			Enhancer	199	914	139	97	354	15.8
			Control	180	912	153	85	338	17.5
	High tannin	0 h	Inhibitor	163	902	139	130	348	18.2
			Enhancer	183	924	122	173	339	18.7
			Control	162	916	130	130	329	19.7
		24 h	Inhibitor	196	917	137	120	380	16..6
			Enhancer	204	919	134	136	354	16.4
			Control	201	905	137	113	333	18.7
Significance	Main effects		s.e.d.	10.3	7.5	7.1	23.4	13.7	1.49
			P	0.683	0.056	0.946	0.957	0.480	0.830
			P legume (L)	<0.001	<0.001	<0.001	0.361	<0.001	<0.001
			P tannin (T)	0.070	0.012	0.666	0.079	0.192	<0.001
			P wilt (W)	<0.001	0.247	0.063	0.015	0.181	<0.001
			P additive (A)	0.109	0.321	0.105	0.004	<0.001	0.148
			P LxT	0.874	<0.001	<0.001	<0.001	0.172	0.041
			P LxW	<0.001	0.891	0.315	0.998	0.077	0.113
			P LxA	0.022	0.016	0.109	0.167	0.567	0.042
			P TxW	0.009	0.397	0.819	0.787	0.877	0.860
			P TxA	0.990	0.663	0.651	0.484	0.620	0.941
			P WxA	0.488	0.970	0.504	0.202	0.571	0.540
			P LxTxW	0.815	0.950	0.939	0.730	0.367	0.501
			P LxTxA	0.817	0.339	0.888	0.719	0.556	0.833
			P LxWxA	0.803	0.601	0.077	0.279	0.661	0.370
			P TxWxA	0.293	0.167	0.036	0.208	0.898	0.710

3.3.3.3 Effect of tannin level, additive type and wilting on the nutritive value of whole-crop peas and beans after 120 d ensiling

Table 3.7 presents the effects of tannin level, wilting and additive type on the *in vitro* DM, OM degradation and estimated ME of whole-crop pea and bean silages after 120 d ensiling. Peas had a higher (P<0.001) IVDMD compared to the beans with a value of 0.816 g g^{-1} compared to 0.765 g g^{-1} (s.e.d.= 0.0063). The use of the inhibitor led to a lower (P<0.050) IVDMD of 0.776 g g^{-1} compared to 0.797 g g^{-1} for the enhancer and 0.798 g g^{-1} for the control (s.e.d.= 0.0076). The high tannin bean silage had a lower (P<0.050) IVDMD compared to the low tannin bean silage (0.750 and 0.798 g g^{-1} respectively, s.e.d.= 0.0088) with no difference being observed between the high and low tannin cultivars of peas.

The peas had a higher (P<0.001) IVDOMD compared to the beans (0.816 and 0.751 respectively, s.e.d.= 0.0063). Addition of the inhibitor additive resulted in a lower (P<0.050) IVDOMD than either the enhancer or control (0.768, 0.791 and 0.791 g g^{-1} respectively, s.e.d.=0.0077). There was a legume x tannin interaction with the low tannin beans having a higher IVDOMD (P<0.050) than the high tannin beans (0.767 compared to 0.734 g g^{-1}, s.e.d.= 0.0063), with no difference being observed between high and low tannin varieties of peas.

Estimated ME (using NCD concentration) was highest (P<0.001) in the peas, compared to the beans (12.2 and 11.0 MJ kg^{-1} DM respectively, s.e.d.=0.06). The inhibitor treated silages had the lowest (P<0.050) estimated ME and the control the highest, with an intermediate value for the enhancer (11.5, 11.8 and 11.6 MJ kg^{-1} DM respectively, s.e.d.= 0.07). There was no effect of wilting or tannin level on estimated ME.

Table 3.7 Effect of tannin content, wilting and additive type on the *in vitro* DM (IVDMD) and OM (IVDOMD) degradation and estimated metabolisable energy (ME) of whole-crop pea and bean silages after 120 d ensiling

Legume	Tannin	Wilt	Additive	IVDMD (g g^{-1} DM)	IVDOMD (g g^{-1} OM)	Estimated ME† (MJ kg^{-1} DM)
Pea	Low tannin	0 h	Inhibitor	0.805	0.802	12.3
			Enhancer	0.853	0.853	12.2
			Control	0.807	0.805	12.3
		24 h	Inhibitor	0.806	0.805	12.2
			Enhancer	0.800	0.800	12.2
			Control	0.805	0.805	12.6
	High tannin	0 h	Inhibitor	0.813	0.817	12.0
			Enhancer	0.835	0.834	12.1
			Control	0.834	0.834	12.4
		24 h	Inhibitor	0.792	0.794	12.3
			Enhancer	0.819	0.819	12.4
			Control	0.824	0.825	12.2
Bean	Low tannin	0 h	Inhibitor	0.787	0.775	11.0
			Enhancer	0.756	0.741	11.1
			Control	0.791	0.778	11.0
		24 h	Inhibitor	0.749	0.735	10.8
			Enhancer	0.779	0.769	11.0
			Control	0.818	0.808	11.0
	High tannin	0 h	Inhibitor	0.746	0.715	11.0
			Enhancer	0.757	0.743	11.1
			Control	0.749	0.735	11.3
		24 h	Inhibitor	0.713	0.698	10.8
			Enhancer	0.780	0.769	11.0
			Control	0.753	0.742	11.2
Significance		Main effects	s.e.d.	0.0022	0.0022	0.20
			P	0.583	0.345	0.376
			P legume (L)	<0.001	<0.001	<0.001
			P tannin (T)	0.066	0.051	0.800
			P wilt (W)	0.215	0.411	0.897
			P additive (A)	0.008	0.002	0.008
			P LxT	0.004	<0.001	0.242
			P LxW	0.154	0.084	0.077
			P LxA	0.426	0.299	0.827
			P TxW	0.889	0.999	0.845
			P TxA	0.345	0.285	0.656
			P WxA	0.205	0.235	0.954
			P LxTxW	0.725	0.942	0.713
			P LxTxA	0.048	0.033	0.346
			P LxWxA	0.028	0.040	0.739
			P TxWxA	0.499	0.542	0.216

† Estimated using the equation of Givens (1989)

3.3.3.4 Effect of tannin level, additive type and wilting on the nutritive losses of whole-crop peas and beans after 120 d ensiling

The loss of DM in the maxi silos was unaffected by legume type or tannin level (Table 3.8). Wilting for 24 h reduced the mean DM loss from 0.095 to 0.078 kg kg^{-1} whilst the fermentation inhibitor had the greatest DM loss (P<0.050) of 0.117 kg kg^{-1} compared to a mean of 0.071 kg kg^{-1} for the control and enhancer treated silages. There was an interaction between legume and wilting, with wilting the bean silages for 24 h resulting in a reduced (P<0.050) DM loss (from 0.097 to 0.065 kg kg^{-1}) with no difference being observed between the 0 and 24 h wilted peas. The interaction between legume type and additive was due to the inhibitor treated silages having the highest (P<0.050) DM loss in both legumes, but the lowest pea DM loss was recorded in the control treatment, whereas the lowest DM loss was recorded in the enhancer treated beans.

There was no difference in OM loss between either legume type or due to tannin content. However, wilting for 24 h reduced (P=0.002) the OM loss by 0.024 kg kg^{-1}, and the use of the fermentation inhibitor resulted in the greatest (P<0.050) loss of 0.131 kg kg^{-1} compared to a mean of 0.086 kg kg^{-1} for the enhancer and control. There was an interaction (P=0.001) between legume type and additive, with the fermentation inhibitor having the greatest loss across both legumes, and the lowest losses being recorded for the enhancer treated peas, and the control treatment in the beans. The proportion of WSC remaining after 120 d ensiling was greatest (P<0.001) in the pea silages, with a value of 0.257 kg kg^{-1} compared to 0.180 kg kg^{-1} remaining for the bean silages. There was no effect of any treatment on the ensiling losses of CP, starch or NDF.

Table 3.8 Effect of tannin level, wilting and additive type on the loss of dry matter, organic matter and water soluble carbohydrates of whole-crop peas and beans ensiled for 120 d

Legume	Tannin	Wilt	Additive	Proportion loss (kg kg^{-1}) DM	OM	WSC
Pea	Low tannin	0 h	Inhibitor	0.099	0.122	0.766
			Enhancer	0.103	0.130	0.732
			Control	0.073	0.082	0.771
		24 h	Inhibitor	0.136	0.144	0.802
			Enhancer	0.102	0.122	0.719
			Control	0.050	0.064	0.717
	High tannin	0 h	Inhibitor	0.119	0.141	0.763
			Enhancer	0.084	0.105	0.700
			Control	0.076	0.099	0.677
		24 h	Inhibitor	0.104	0.113	0.728
			Enhancer	0.106	0.108	0.762
			Control	0.049	0.050	0.776
Bean	Low tannin	0 h	Inhibitor	0.098	0.115	0.854
			Enhancer	0.055	0.072	0.787
			Control	0.079	0.100	0.840
		24 h	Inhibitor	0.126	0.136	0.791
			Enhancer	0.024	0.033	0.820
			Control	0.050	0.062	0.840
	High tannin	0 h	Inhibitor	0.174	0.184	0.851
			Enhancer	0.063	0.082	0.781
			Control	0.114	0.124	0.833
		24 h	Inhibitor	0.077	0.091	0.835
			Enhancer	0.055	0.063	0.797
			Control	0.056	0.078	0.817
Significance		Main effects	s.e.d.	0.0253	0.0263	0.0531
			P	0.594	0.460	0.156
			P legume (L)	0.147	0.134	<0.001
			P tannin (T)	0.346	0.549	0.522
			P wilt (W)	0.027	0.001	0.790
			P additive (A)	<0.001	<0.001	0.160
			P LxT	0.141	0.104	0.651
			P LxW	0.035	0.142	0.447
			P LxA	0.002	0.001	0.675
			P TxW	0.066	0.064	0.361
			P TxA	0.921	0.837	0.955
			P WxA	0.247	0.444	0.501
			P LxTxW	0.279	0.718	0.449
			P LxTxA	0.966	0.813	0.557
			P LxWxA	0.754	0.791	0.851
			P TxWxA	0.011	0.037	0.560

3.3.3.5 Effect of tannin level, additive type and wilting on the in vitro rumen fermentation kinetics of whole-crop peas and beans after 120 d ensiling

Since the underlying rate (b) and time dependent rate (c) are used in the calculation of the fractional rate of degradation (μ; Equation 3.6), they will not be described in any detail. Peas had a higher (P<0.010) asymptote than beans (303 and 294 ml g^{-1} DM respectively, s.e.d.= 3.4, Table 3.9) and a faster (P<0.010) μ (0.064 and 0.061 h^{-1} respectively, s.e.d.= 0.0010, Table 3.9). This can be seen graphically in Figure 3.13.

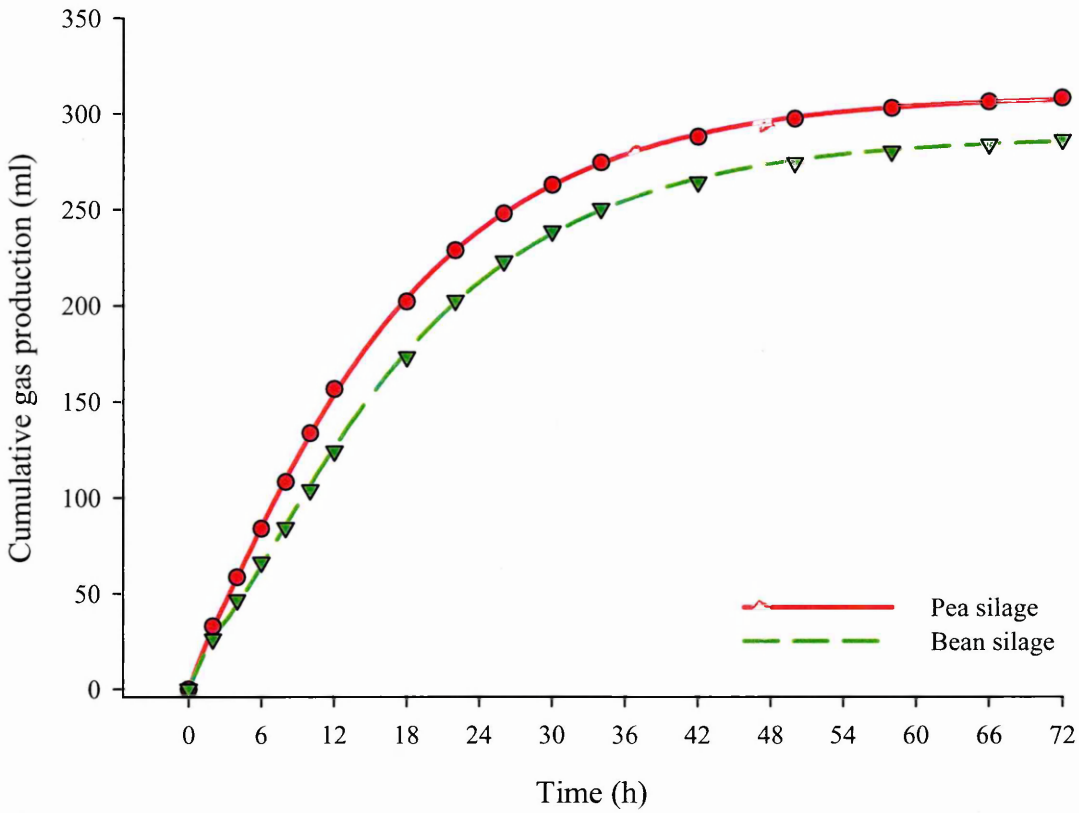


Figure 3.13 Cumulative gas production profiles of whole-crop pea and bean silages, per gram DM, fitted to the model of France et al. (1993, lines), symbols represent actual values

Wilting for 24 h decreased (P<0.050) the asymptote from 303 to 294 ml g^{-1} DM (Table 3.9). The inhibitor and control treatments had identical asymptotes of 304 ml g^{-1} DM whereas the enhancer had a lower (P<0.001) asymptote of 288 ml g^{-1} DM. Figure 3.14 shows the interaction between legumes and additive type, with the highest asymptote being recorded by the enhancer treated peas (309 ml g^{-1} DM, Table 3.9) and the lowest in the enhancer treated beans (274 ml g^{-1} DM, Table 3.9). The inhibitor treated silages had the

lowest (P<0.050) μ of 0.060 compared to a mean of 0.063 for the enhancer and control treated silages (s.e.d.=.0012). There was an additive x wilt interaction (P=0.001), caused by the inhibitor treated silages having the highest asymptote when wilted for 0 h, whereas the asymptote was highest in the control treated silages after a 24 h wilt.

Pea silages lag time of 25 min was 2.92 fold greater (P<0.001) than the 73 min recorded for the bean silages (s.e.d.= 4.1). The high tannin cultivars started to ferment after a lag time of 43 min, some 13 min earlier than the low tannin varieties. There was no effect of wilting on the bean silages, with a mean lag period of 73.5 min (Table 3.9). However, wilting the peas for 24 h reduced (P<0.001) the lag time by half, with a lag period of 17 min. The estimated rumen degradability (extent), at an outflow rate of 0.02 h^{-1}, was affected by legume type, with peas being more (P<0.001) degradable than beans (0.602 compared to 0.536 g g^{-1}, s.e.d.= 0.0082), and additive type, with the inhibitor resulting in a lower (P<0.050) degradability compared to the enhancer and control (0.552, 0.575 and 0.581 g g^{-1} respectively, s.e.d.= 0.0082). The same statistical trends were seen when calculating the estimated rumen degradability at the higher rumen outflow rates of 0.05 and 0.08 h^{-1}. Regression equations were calculated for legume types and additive types and are presented below.

$$Extent_{Pea} = -4.7833k + 68.47 \ (r^2 = 0.9761) \qquad \text{Equation 3.7}$$

$$Extent_{Bean} = -4.7833k + 61.83 \ (r^2 = 0.9731) \qquad \text{Equation 3.8}$$

$$Extent_{In} = -4.7133k + 63.28 \ (r^2 = 0.9734) \qquad \text{Equation 3.9}$$

$$Extent_{En} = -4.8067k + 65.76 \ (r^2 = 0.9751) \qquad \text{Equation 3.10}$$

$$Extent_{Co} = -4.8300k + 66.42 \ (r^2 = 0.9753) \qquad \text{Equation 3.11}$$

Where k = rumen outflow rate, In = inoculant, En = enhancer and Co = control

Table 3.9 Effect of tannin level, wilting and additive type on the gas production parameters; asymptote (A), underlying rate (b), time dependent rate (c), fractional rate of fermentation (μ), discrete lag (T) and estimated rumen degradation (extent); of whole-crop pea and bean silages ensiled for 120 d

Legume	Tannin	Wilt	Additive	A† (ml)	b (h^{-1})	c ($h^{-1/2}$)	μ (h^{-1})	T (h)	Extent‡ ($g\ g^{-1}$)
Pea	Low tannin	0 h	Inhibitor	329	0.078	-0.108	0.063	0.490	0.587
			Enhancer	318	0.083	-0.141	0.063	0.740	0.616
			Control	286	0.086	-0.170	0.063	1.015	0.590
		24 h	Inhibitor	295	0.077	-0.097	0.063	0.400	0.596
			Enhancer	311	0.076	-0.095	0.063	0.405	0.587
			Control	296	0.074	-0.074	0.063	0.255	0.595
	High tannin	0 h	Inhibitor	307	0.076	-0.090	0.054	0.370	0.600
			Enhancer	286	0.085	-0.101	0.062	0.365	0.629
			Control	314	0.082	-0.100	0.068	0.375	0.626
		24 h	Inhibitor	306	0.068	-0.057	0.060	0.175	0.580
			Enhancer	295	0.073	-0.069	0.063	0.225	0.606
			Control	299	0.075	-0.070	0.065	0.230	0.613
Bean	Low tannin	0 h	Inhibitor	324	0.085	-0.217	0.057	1.620	0.533
			Enhancer	263	0.082	-0.168	0.059	1.035	0.517
			Control	298	0.092	-0.198	0.064	1.185	0.567
		24 h	Inhibitor	280	0.088	-0.201	0.061	1.315	0.526
			Enhancer	269	0.091	-0.201	0.063	1.230	0.557
			Control	307	0.090	-0.215	0.061	1.440	0.573
	High tannin	0 h	Inhibitor	315	0.075	-0.159	0.054	1.135	0.503
			Enhancer	281	0.091	-0.211	0.062	1.360	0.534
			Control	317	0.090	-0.168	0.066	0.880	0.552
		24 h	Inhibitor	279	0.079	-0.176	0.060	1.235	0.491
			Enhancer	281	0.087	-0.186	0.062	1.145	0.554
			Control	313	0.084	-0.172	0.061	1.090	0.532
Significance			s.e.d.	11.7	0.0055	0.0273	0.0033	0.2388	0.0023
			P	0.455	0.480	0.172	0.990	0.171	0.747
	Main effects		*P* legume	0.009	<0.001	<0.001	0.005	<0.001	<0.001
			P tannin	0.672	0.058	0.002	0.717	0.005	0.763
			P wilt	0.015	0.037	0.030	0.322	0.098	0.578
			P additive	<0.001	0.011	0.618	0.004	0.910	0.004
			P LxT	0.085	0.728	0.469	0.221	0.486	0.027
			P LxW	0.453	0.014	0.007	0.306	0.031	0.240
			P LxA	<0.001	0.797	0.731	0.384	0.234	0.258
			P TxW	0.751	0.239	0.849	0.070	0.436	0.269
			P TxA	0.057	0.113	0.306	0.090	0.343	0.246
			P WxA	0.001	0.252	0.733	0.304	0.996	0.846
			P LxTxW	0.424	0.697	0.318	0.679	0.371	0.876
			P LxTxA	0.034	0.649	0.292	0.989	0.307	0.394
			P LxWxA	0.222	0.758	0.422	0.193	0.168	0.134
			P TxWxA	0.074	0.499	0.455	0.792	0.508	0.889

† parameter estimate, ‡ estimated rumen degradability at an outflow rate of 0.02 h^{-1}

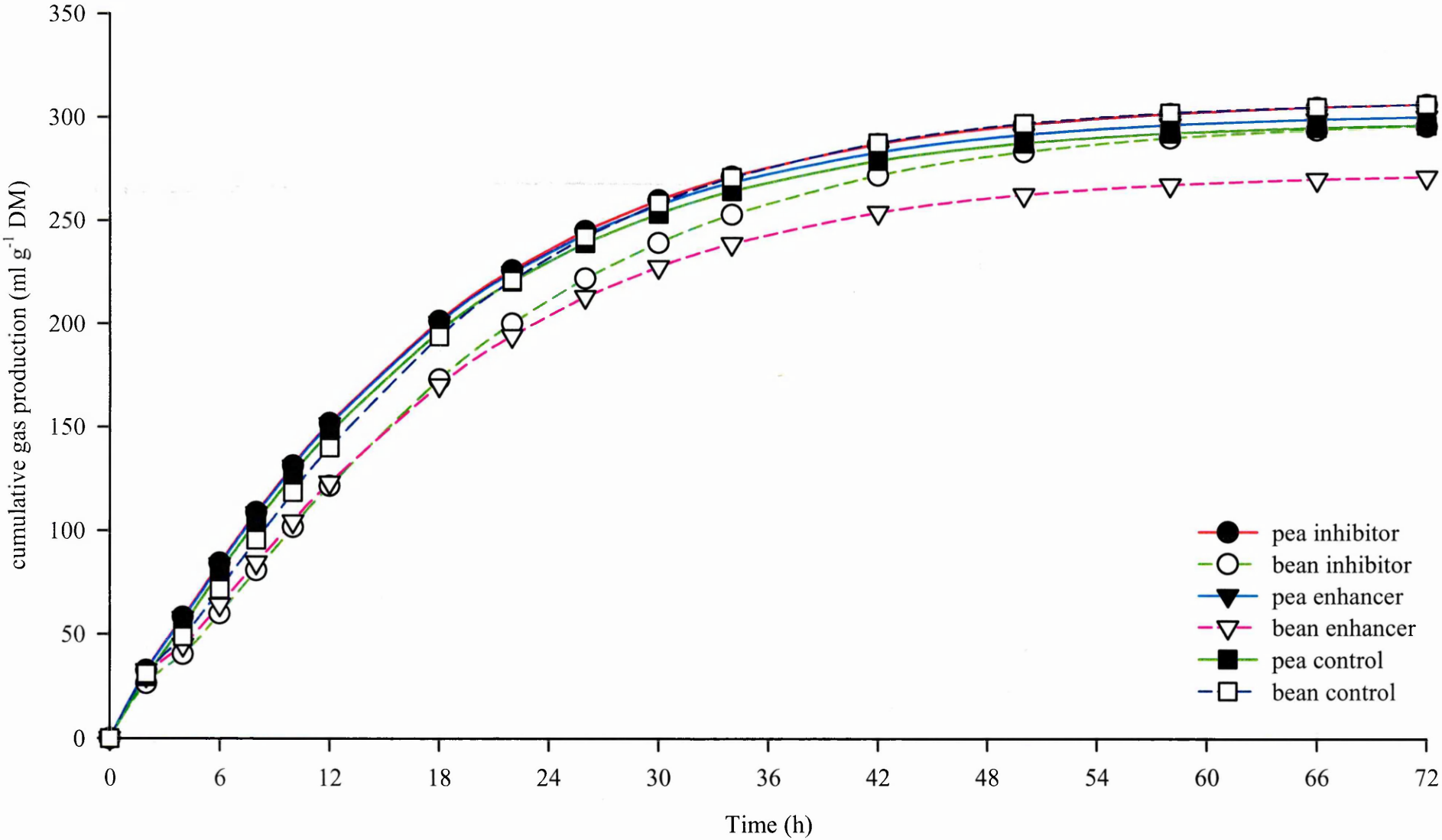


Figure 3.14 Gas accumulation profiles of ensiled whole-crop peas and beans treated with different additives

3.4 Discussion

The peas and beans used in this experiment were grown as a commercial crop and as a result it was not possible to accurately determine yields. The use of plastic bags as time point silos proved successful, with fermentation following the expected pattern. The use of bags also allowed for increased replication of treatments and a larger capacity compared to boiling tubes and 1 l glass jar laboratory silos, which have been used in other ensiling studies (e.g. Winters and Minchin, 2002).

The CP content of the pea silages was within the expected range, whereas the value of 126 g kg^{-1} DM recorded in the beans was lower than expected. Faulkener (1985) concluded that beans grown as a pure stand were a more robust crop than a pure stand of peas, and would therefore be a more practical forage crop. Observations made in this study do not support this conclusion, as neither the peas nor beans lodged, prior to harvest, and there was no difference in DM yield. The use of pure stands of legumes would be of benefit to organic farmers, and farmers with land in nitrate vunerable zones, due to their N fixing capability (Anil *et al.*, 1998), thus reducing the requirement for N fertiliser in both the current and future crops in the rotation.

3.4.1 Initial crop chemical composition

3.4.1.1 Choice of varieties

The different varities of peas and beans evaluated in this study were chosen to differ only in flower colour, indiciating tannin level (Section 1.2.2.2), and to have similar morphological and disease resistance characteristics. Crop yield was not used as a selection criterium since only grain yields were reported by NIAB (2001). It is acknowledged that crop variety and tannin content are confounded due to the crops not being near isogenic lines.

3.4.1.2 Chemical composition

The chemical composition of the initial, unwilted, untreated, peas and beans supports the assumption that there was little difference in terms of nutritive value between different cultivars in terms of OM, CP, NDF and WSC content. There was, however, a difference in tannin and starch content, with the low tannin varieties having a higher starch content and lower tannin content (95.2 g starch kg^{-1} DM and 13.1 g TAE kg^{-1} DM respectively) compared to the higher tannin varieties (62.4 g starch kg^{-1} DM and 34.8 g TAE kg^{-1} DM respectively). The difference in levels of condensed tannin (CT) between varieties (i.e. 1.8 fold and 3.9 fold for peas and beans respectively) obtained in this study were less than the 4.8 fold and 10.8 fold difference obtained by Griffiths (1981). However, in contrast to this study, Griffiths (1981) compared the CT content of the seed and not the whole plant. The observed ratio between high and low tannin varieties for beans was approximately twice that of the peas in both the current study and that of Griffiths (1981).

Fraser *et al.* (2001) reported that peas responded better to wilting than beans, with an increase in DM of 200 and 57 g kg^{-1} FM respectively. Results from this current study gave rise to a difference of only 52 and 32 g kg^{-1} FM for peas and beans respectively. The work by Fraser *et al.* (2001) utilized a forced wilt, undercover, using a forced draught drier delivering ambient temperature air at a velocity of 0.35 m s^{-1}. In the current study, the environmental conditions to which the crops were subjected during wilting differed in mean temperature of 21.2 vs. 16.7°C, mean wind speed of 2.1 vs. 1.2 m s^{-1}, and relative humidity of 73.3 vs. 76.3%, for peas and beans respectively. There was no rainfall recorded on either harvest date. A potential reason for this difference in response to wilting may be due to the difference in morphological composition of peas and beans. The beans had a thicker stem compared to the peas, therefore decreasing the surface area to volume ratio. Fraser *et al.* (2001) reported that the stem accounted for (on a proportion of DM basis) approximately 0.25 in peas compared to approximately 0.45 for beans. Conditioning

of the crop at harvest would increase the rate of evaporation, and hence increase the DM content (McDonald *et al.*, 1991). However, the mechanical action of conditioning peas and beans may result in an increased loss of leaves and pods, and hence, potentially reduce their subsequent nutritive value (McDonald *et al.*, 1991).

The DM content of the wilted peas and beans in this study (282 and 205 g kg^{-1} FM respectively) was comparable to the values reported by other authors at the same growth stage (Table 3.10). The main differences in the nutritive value observed here, and in other studies, were that the peas used here had a significantly lower NDF content (Table 3.10). This was probably due to the difference in plant morphology between forage varieties (tall and leafy), relying on their stems to remain erect, and semi-leafless combinable types (short stems), which rely on the entanglement of their tendrils to remain erect (Koivisto *et al.*, 2002). The CP content of the beans used in this study was surprisingly low when compared to similar varieties quoted in the literature.

Table 3.10 Chemical composition and growth stage (GS) of wilted whole-plant peas and beans prior to ensiling

Crop	DM	CP	WSC	Starch	NDF	GS	Reference
Peas	354	157	118	75	419	205	Fraser *et al.* (2001)
	285	188	nr	93	381	205‡	Mustafa *et al.*† (2002b)
	282	**156**	**80**	**71**	**217**	**205**	**Current study**
Beans	210	180	97	64	376	207	Fraser *et al.* (2001)
	183	172	124	nr	339	207‡	Ghanbari-Bonjar and Lee (2003)
	205	**123**	**61**	**93**	**300**	**207**	**Current study**

nr not reported, ‡ approximate GS from crop description, †mean of 3 varieties

In a field situation it would be unwise to harvest crops for ensilage at less than 250 g kg^{-1} FM due to the amount of effluent that is likely to be produced. In the current study, the wilted beans did not achieve this value. It would therefore make more sense to harvest beans at a later growth stage, which agrees with the recommendations of Fraser *et al.* (2001). Harvesting at a later maturity would further decrease the WSC content of the

beans, making them difficult to ensile, and it may be advisable to take them through to senescence and harvest the grain and straw separately. Lanza *et al.* (1999) demonstrated that faba bean meal can directly replace soya-bean meal in the diets of growing lambs.

3.4.2 Ensiling profile

The inhibitor used in this study falls into two classes of additive; firstly, it is an acid designed to drop the pH of the herbage mass and secondly, it has antimicrobial (formaldehyde) properties designed to inhibit microflora in general (Woolford and Pahlow, 1998). The application of the fermentation inhibitor resulted in an initial drop in pH to pH 5.0, compared to an initial value of pH 5.6 for both the enhancer and the control (Figure 3.17). In a study by Carpintero *et al.* (1979), the application of 4.1 g kg^{-1} FM formic acid (85%) to herbage from a ryegrass clover sward resulted in an immediate drop in pH to 4.1 compared to the control value of pH 5.9. Fraser *et al.* (2001) reported a buffering capacity (BC) of 234 and 341 (mequiv. 100 g^{-1}) for peas and beans respectively, harvested at growth stage 205 and 207 repetitively, whereas Playne and McDonald (1966) reported a value of 70 (mequiv. 100 g^{-1}) as a typical value for a grass clover herbage. It is possible that due to the high BC of legumes (Playne and McDonald, 1966), the application rate of 4 l t^{-1} FM of formic acid and formaldehyde was not sufficient.

Work by Mustafa *et al.* (2002) looked at the change in different fractions of CP during ensiling of whole-crop pea silage and reported a reduction in true protein (TP) content of approximately 11% and an increase in non protein nitrogen (NPN) of approximately 1.13 fold over the first 2 d of ensiling, with no change at 4, 8, 16 and 70 d post ensiling. The extent to which TP is reduced to NPN and NH_3-N is related to the activity of the plant proteases and the rate of microbial growth respectively (Ohshima and McDonald, 1978). However, both are dependant on the pH of the ensiling mass (McDonald *et al.*, 1991). Work by Carpintero *et al.* (1969) concluded that silage NH_3-N content was correlated with pH, but their equation was limited to unwilted herbage. The difference between the mean NH_3-N of peas and beans at 0 h post ensiling (Figure 3.7) may be explained by a difference between legume types. Alternatively, it could simply be related to the time taken to freeze beans in relation to peas due to the difference in DM, and hence delay the cessation of

proteolytic activity of both enzymic and microbial activity. The extent of NH_3-N formation due to the different additives seems inversely proportional to the pH of the ensiling mass, with the fermentation enhancer and control treatments following the same pattern, i.e. supporting extensive bacterial growth, whereas the fermentation inhibitor, although not completely inhibiting bacterial growth, slowed the proliferation of lactic acid bacteria (Figure 3.4 and 3.10).

Although tannin level did not affect pH, high tannin levels reduced the extent of protein catabolism and lowered the mean NH_3-N content in both peas and beans. This effect of tannin was in agreement with Albrecht and Muck (1991), who found that tannins played a major role in limiting proteolysis when comparing high and low tannin isolates of Sericea lespepeza and Birdsfoot trefoil, although other factors were suggested to be involved.

Virtanen (1933) concluded that a pH of 4.0 was the decisive limit of acidity after which proteases were inhibited. Macpherson (1952a) reported that a pH of 4.3 prevented proteolysis during ensiling, although this work used only grass sap as the substrate. More recently, McKersie (1985) studied the optimum pH conditions for proteases of alfalfa, red clover and birdsfoot trefoil *in vitro* and reported values of pH 6.0, 6.5 and 6.5 respectively, and concluded that proteolytic activity was dramatically reduced at a pH below 4.5. The optimum pH for the activity of ryegrass proteases has been determined to be between pH 5 and 7 but work by Heron *et al.* (1989) narrowed this down to be closer to a pH of 6. Using Macpherson's (1952a) cut off point of pH 4.3, results from this study would suggest that, on average, beans achieved this between 48 and 96 h whereas peas reached it after 240 h (Figure 3.1). The mean effect of additive was to achieve a pH of 4.3 after 48 h for the enhancer, 96 h for the control and the inhibitor treatment not reaching it after 240 h (Figure 3.4). It can therefore be assumed that proteolysis occurred in all treatments over most of the 240 h examined (Table 3.3), but it is assumed that it would plateau out soon after this time, in agreement with the study by Mustafa *et al.* (2002), who found proteolysis did not

occur after 16 d. In order to achieve a pH of less than pH 4.3, it can be concluded that an increased application rate of the fermentation inhibitor was required, e.g. 5 or 6 l t^{-1} FM. This would, however, add to the cost of production and may increase potential health problems associated with handling and storing such products. Due to the integrity of the silos used, with no seepage being observed, the change in DM over the first 240 h of ensiling was small (Figure 3.12). This is similar to work by Raut and Varade (1998), and Ashbell *et al.* (2001), who reported DM losses of 0.030 and 0.011 g g^{-1} respectively for silages made in 'plastic bags' preserved for over 28 d. It can therefore be assumed that the loss in DM was ascribable to gaseous loss, caused by the respiration of the plant material, i.e. $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2870$ kJ (McDonald *et al.*, 1991), during the initial aerobic phase of ensiling (Pitt *et al.*, 1985).

Examining the ensiling profiles of both peas and beans, it is evident that both crops ensiled well, with an acidic pH being achieved quickly and a low proportion of NH_3-N. The use of additives in this study has shown that overall the enhancer and control behaved similarly during the first 240 h of ensiling. The fermentation inhibitor reduced the mean NH_3-N formation but lowered the achievement of a stable pH, which in turn may benefit micro-organisms that are detrimental to the stability of silage. Bacterial based silage inoculants are easy to handle and store, and many are acceptable for use in organic systems. The high tannin varieties of both peas and beans reduced the breakdown of CP to NH_3-N. It is unclear whether the levels of CT found in this study would affect animal performance.

3.4.3 Final silage

There were very few yeast and visible mould growths observed on the top of the silages during opening of the maxi silos. Following opening, the ensiled mass of beans in the maxi silos rapidly changed colour, from dark green to black, an effect also reported by Ingalls *et al.* (1979). This may be attributable to the enzyme polyphenol oxidase (Winters and Minchin, 2002), but can not be confirmed without further study.

3.4.3.1 Ensiling characteristics and nutritive losses

McDonald and Edwards (1976) broadly classified silages into 5 categories; lactate, acetate, butyrate, wilted and chemically restricted. The mean results from this study (Tables 3.5 and 3.6) fit the description of a lactate silage. The lactate silage is characterised by a low pH (3.7-4.2) low NH_3-N (<120 g kg^{-1} TN) and low residual WSC content (<20 g kg^{-1} DM). The mean pH, NH_3-N and WSC content of all the final silages was pH 4.1, 79.3 g kg^{-1} TN and 17.0 g kg^{-1} DM respectively. The mean pH of all the silages was very similar at pH 4.1 which, when compared to peas and beans ensiled at the same growth stage, was similar to the mean of pH 4.2 for peas, but higher than the mean of pH 3.7 for beans (Tables 1.9 and 1.10). The pH after 120 d ensiling was less than that of pH 4.3 recorded after 240 h ensiling, indicating crops were still undergoing fermentation after 10 d (McDonald *et al.*, 1991). With the application of LAB to pea and bean silages, Fraser *et al.* (2001) reported a more acidic pH of 3.7 vs. 4.0 and pH 3.6 vs. 3.8 for enhancer and control treated, peas and beans respectively.

After 120 d there was no longer any difference in NH_3-N content of the ensiled peas and beans, with mean values of 76.8 and 81.7 g kg^{-1} TN respectively, compared to the difference at 240 h post ensiling (Figure 3.7). The concentration of NH_3-N was lowest in the fermentation inhibitor treated crops, a finding in agreement with that of Henderson

(1993), who reported no difference between the control and enhancer treatments, but the inhibitor reduced NH_3-N content by 21 g kg^{-1} TN.

Increasing the DM of the peas by wilting for 24 h resulted in a higher NH_3-N concentration compared to wilting for 0 h (80.6 and 72.9 g kg^{-1} TN respectively). This is in contrast to the beans and to the findings of Hristov and Sandev (1998), who found increasing the DM of a crop pre-ensiling resulted in a lower NH_3-N concentration. The role of tannins in protecting proteins was still evident at 120 d post ensiling for the same reasons as summarised earlier (Section 3.4.2). The residual WSC content of the silages was low (<27 g kg^{-1} DM for peas and <17 g kg^{-1} for beans). This is in agreement with the whole-crop pea silage produced by Salawu *et al.* (2002b), which contained a residual WSC of 16.4 g kg^{-1} DM and the untreated bean silage produced by Fraser *et al.* (2001), with a residual WSC of 16.0 g kg^{-1} DM.

Mean silage DM loss was not affected by effluent losses, as the integrity of the plastic bag lined silos inhibited effluent escape. Work by Mayne and Gordon (1986) grouped DM losses into three categories; surface waste, effluent and invisible, and they reported that wilting herbage prior to ensilage reduced effluent and invisible losses of DM by approximately half, whereas in this experiment wilting for 24 h reduced total DM loss by approximately one fifth (0.179 kg kg^{-1}). The higher DM losses observed in the inhibitor treated silages may have been due to a change in the microflora population caused by the immediate lowering of the pH and the sterilant activity of the formalin. The silages produced in this study may not, however, truly represent what may happen in a clamp situation, in which effluent leeching may occur. Since there was no difference between additives, in the proportion of WSC lost during ensiling, it would have been expected that the resultant pH would be the same. However, inhibitor treated silages had a higher pH, potentially indicating a less efficient WSC utilisation during fermentation.

3.4.3.2 Effect of tannin level, additive type and wilting on the chemical composition and nutritive value of whole-crop peas and beans after 120 d ensiling

In a review of silage additives, Henderson (1993) regarded wilting to a DM of 250-300 g kg^{-1} FM as an alternative to additive application. In this study, only the 24 h wilted peas, with a mean DM of 259 g kg^{-1} FM, fitted into this category. The mean DM of the pea and bean silages (236 and 182 g kg^{-1} FM respectively), are at the lower end of the range of 153-528 and 165-370 g kg^{-1} FM reported in the literature (Tables 1.9 and 1.10). The mean CP of the bean silages (138 g kg^{-1} DM) is somewhat lower than that reported by other authors (155-225 g kg^{-1} DM, Table 1.9), whereas the mean CP for the pea silages is in the range (158-228 g kg^{-1} DM) reported in the literature (Table 1.10). The CP results of the final silages were similar to those reported at 240 h post ensiling. However, the concentration of NH_3-N increased from a mean of 46.0 g kg^{-1} TN at 240 h post ensiling to 79.3 g kg^{-1} TN after 120 d ensiling.

Residual starch concentrations were in excess of 50 g kg^{-1} DM higher than those reported by Fraser *et al.* (2001), for pea and bean silages produced at the same growth stage. The difference in NDF contents of the peas and beans has been discussed earlier (Section 3.4.1). The effect of additives on NDF was unexpected; the inhibitor had the highest NDF and the control treatment the lowest. It was expected that the enhancer would have had the lowest NDF because of the inclusion of cellulytic enzymes within the additive as these would have bean expected to have hydrolysed the structural carbohydrates to simple sugars. Work by Ranjit and Kung (2000) found that the application of *Lactobacillus buchneri* (LB) dropped the mean NDF of corn silage from 462 in the control to 448 g kg^{-1} DM, and in a further study, Kung and Ranjit (2001) reported similar results with barley silages, with the NDF decreasing from 685 to 672 g kg^{-1} DM.

The main effect of treatment on IVDMD and IVDOMD was the difference between the two legumes, with peas being more degradable than beans. The mean *in vivo* DOMD reported by Fraser *et al.* (2001) was significantly lower than those obtained in this study. However, the results of Kristensen (1992) were similar to those obtained here. A mean *in vivo* DMD of 0.713 has been reported by Mustafa *et al.* (2002) for whole-crop pea silage, which is lower than that obtained in the peas in this study. The effect of additive observed in this study, i.e. the inhibitor resulting in the lowest and the enhancer and control being the same, was in contrast to the findings of Nadeau *et al.* (2000), who found that formic acid treated silages had a higher IVDMD compared to control or inoculated silages.

Both peas and beans had a mean IVDOMD in excess of 0.75, which makes them comparable to a good quality grass silage (AFRC, 1993). The highest level of CT was recorded in the high tannin variety of beans, which may have resulted in the lower degradation characteristics recorded here, although there was no difference between high and low tannin varieties of peas. The silages treated with the inhibitor had the lowest estimated ME, with the control treated silages having the highest, although this estimate was made using a predictive equation utilising NCD and using an equation formulated for dried lucerne. The ME obtained in this study for pea and beans silages were higher than those of 11.3 and 10.5 MJ kg^{-1} DM reported by Kristensen (1992), although the method of determination was not stated.

When the mean chemical composition of the peas and beans was compared, it was clear that the peas had a potentially better nutritive composition, with a higher DM, CP, ME and WSC and a lower NDF and NH_3-N content. The higher tannin levels reduced proteolysis across both crops, as did the use of the fermentation inhibitor.

3.4.3.3 Effect of tannin level, additive type and wilting on the fermentation kinetics of whole-crop peas and beans after 120 d ensiling

The gas accumulation profiles obtained in this study where determined using thawed silage samples that were not subjected to any processing, and were hence in the same state as would be offered to an animal. Work by Lowman *et al.* (*In press*) examined the effects of different drying methods (freeze, oven and microwave) compared to fresh grass on the resultant gas accumulation profile. Results from their study indicated that the asymptote was significantly lower in fresh grass compared to the 3 drying methods used, whilst lag time was reduced by freeze drying but increased by oven and microwave drying. These effects may be explained by the difference in the way moisture is lost between different drying methods. Freeze drying lyses the cells thus increasing the availability of the cell contents to the rumen micro-organisms and therefore comparisons made against dried samples would result in the fresh sample appearing less fermentable.

The effect of host diet has been examined by Huntington *et al.* (1998) who concluded that although individual accumulation curves were different, for each diet at the start, there was no statistical difference in overall fermentation stoichiometry. There are no gas production kinetic studies of leguminous silages in the literature that use the same kinetic model as used here (France *et al.*, 1993). Cone *et al.* (1999) reported a maximum gas production of 208 ml g^{-1} OM for grass silage, whereas Lovett *et al.* (2004) reported an asymptote of 246 ml g^{-1} OM for maize silage. Results from this study indicate that both peas and beans are highly fermentable with a mean asymptote of 303 and 294 ml g^{-1} DM respectively. The mean rate term, μ, of 0.0620 h^{-1} was lower than that of 0.0639 h^{-1} for the maize silages analysed by Lovett *et al.* (2004). The effect of additives on fermentation kinetics may be explained by the differences in chemical composition of the silages used. The lag phase of 25 min for the pea silages was dramatically lower than that of 71 min reported in maize silage (Lovett *et al.*, 2004). However, this value was similar to the 73 min obtained with

the bean silages. The estimated extents to which the silages degrade in the rumen were disappointingly low compared to their chemical composition. Equations 3.10 to 3.12 show that inoculant treated silages did not degrade to the same extent as either the enhancer or control treated ones, which may be attributable to the effect of additives on NDF concentration. In terms of rumen kinetics determined *in vitro,* both pea and bean silages were superior to maize and grass silage, with faster rates and greater gas production (Cone *et al.*, 1999; Lovett *et al.*, 2004). The use of the fermentation inhibitor reduced the rate of fermentation and overall extent of degradation, which may have been due to the effect of the additive on the buffer solution, potentially changing the pH, and hence altering microbial degradation. Higher levels of tannin did not seem to affect fermentation kinetics; high tannin varieties had a shorter lag time *in vitro* but no other fermentation characteristics were affected. Wilting the crops for 24 h compared to 0 h did not result in any differences in fermentation kinetics.

3.5 Conclusions

Results from this experiment suggest that the optimum conditions for ensiling beans as a whole-crop are after a 24 h wilt without the use of an additive. Wilting beans increased the DM, decreased the NH_3-N content, and had no affect on pH. The use of the control additive was similar to the use of the enhancer in chemical composition, ensiling losses, estimated ME and extent of degradation. However, the enhancer treated beans had a lower gas production. The use of the fermentation inhibitor in beans reduced NH_3-N content, but did not drop the pH sufficiently to stop proteolysis and it increased DM loss. The benefits of using a fermentation inhibitor or enhancer in beans under these experimental conditions were not distinct from the control. In order to make practical use of beans, an extension of the wilting period may be an option, or harvesting at a later growth stage. The use of high tannin beans reduced IVDOMD and may potentially affect rumen function and animal performance.

The optimum conditions for ensiling peas as a whole-crop determined in this experiment were similar to the beans. The utilisation of a 24 h wilt was much more profound in the peas, resulting in a higher pH, increasing the DM and WSC content and reducing the fermentation lag time by half. The inhibitor had the same effect in peas as it did in beans, increasing DM losses, decreasing NH_3-N content, gas production, IVDOMD and estimated ME. The control and enhancer treatments were very similar in all measured attributes except estimated ME, which was higher in the control treatment. The use of either the enhancer or control would be beneficial in the production of whole-crop pea silages. However, economics would suggest using the control. There was little difference between the high and low tannin varieties of peas used, and further study is needed to assess the effect of tannin in peas on animal production.

In a direct comparison of the pea and bean silages produced, pea silages had a higher DM (236 cf. 182 g kg^{-1} FM), CP, estimated ME (12.3 cf. 11.0 MJ kg^{-1} DM), WSC (22.7 cf. 11.2 g kg^{-1} DM), IVDMD (0.816 cf. 0.765) and IVDOMD (0.816 cf. 0.751). Peas and beans had similar NH_3-N, starch and pH values. However, NDF was highest in the bean silages (347 cf. 257). This suggests that pea silage has a superior chemical composition to the bean silages produced and may therefore be a more suitable ruminant livestock feed.

4.0 EXPERIMENT 2: EFFECT OF WHOLE-CROP PEA SILAGES ON THE GROWTH, CARCASS COMPOSITION AND METABOLISM OF GROWING LAMBS

4.1 Introduction

The results of Experiment 1 indicated that there was little difference in the predicted nutritive value between low and high tannin varieties of whole-crop pea silages, with a high estimated ME (12.3 MJ kg^{-1} DM), *in vitro* organic matter degradability (816 g kg^{-1} DM) and a moderately high crude protein content (177 g kg^{-1} DM). Many studies (McLeod, 1974; Broderick and Albrecht, 1997; Komolong *et al.*, 2001; Min *et al.*, 2003) have shown that condensed tannins (CT) complex with proteins, thus reducing their ruminal degradability and hence increasing the amount of digestible undegradable protein reaching the small intestine. However, Min *et al.* (2003) suggested that diets containing more than 55 g CT kg^{-1} DM tended to reduce the voluntary feed intake, digestibility and growth rate of ruminants. Broderick (1995) suggested that feeding forage legumes containing low levels of CT (<50 g kg^{-1} DM) to ruminants improves N utilisation and can prevent bloat.

The N balance and whole tract digestibility of whole-crop pea silages has been evaluated (Efe Serrano, 1989; Fraser *et al.*, 2001), but their use in diets for growing lambs has received little attention. The objectives of this experiment were firstly, to determine the *in sacco* degradation characteristics of whole-crop pea silages differing in tannin content, and secondly, to determine the effect of feeding whole-crop pea silage as an alternative protein source to soya-bean meal, on the growth, carcass composition and metabolism of growing lambs.

4.2 Material and methods

4.2.1 Silage production

4.2.1.1 Whole-crop pea silage

Prior to drilling, the field site was ploughed, sub-soiled and harrowed according to standard agricultural practice. The field soil type was sandy loam with a pH of 6.7, having phosphorous and potassium indices of 7.3 and 3.7 respectively. The field was split in half to form two, 2 ha plots. The pea varieties, Racer (coloured flowers; high tannin) and Croma (white flowers; low tannin) were sown at 220 and 245 kg ha^{-1} respectively, in accordance with the breeder's specifications, on 22nd April 2002 using a pneumatic seed drill (6m, pneumatic, Accord, Kverneland Group UK Ltd., St. Helens, UK).

A gas-powered bird deterrent boomer was set up next to the site in order to deter bird damage. The field was sprayed on two occasions with herbicides. Cyanazine and pendimethalin (Bullet; 5 l ha^{-1}) was applied pre-emergence, and a mixture of cyanazine (Fortrol; 0.2 l ha^{-1}) and MCPA & MCPB (Trioflex-tra; 1.5 l ha^{-1}) was applied post-emergence. The insecticide cypermethrin (Toppel 10; 0.2 l ha^{-1}) was applied, with post-emergent herbicides, following the discovery of pea and bean weevil (*S. lineatus*.).

In order to determine the DM yield of each crop, six quadrats (0.25 m^2) were cut at random, leaving a 10 cm stubble from each field plot, at 16:00 h on the day preceding cutting. The pea plants were weighed immediately and DM determined (Section 2.1).

The peas were mown at approximately 10:00 h on the 26th July 2002, at growth stage 206 (Knott, 1987) using a front mounted drum mower (Claas Corto 252, Bury St. Edmunds, UK) without a conditioner, and leaving a stubble height of 10 cm. The peas were wilted for 30 h prior to being picked up at approximately 16:00 h and precision chopped (3 cm theoretical chop length; TCL) using a self-propelled forage harvester (Claas Jaguar 800,

Bury St. Edmunds, UK). A fermentation enhancer (bacterial inoculant; Wholecrop Legume, Biotal, Cardiff, UK) was applied by the forage harvester at a rate of 4 l t^{-1} FM. Precision chopped, additive treated material was transferred into 2 separate, plastic lined, concrete walled, roofed bunker silos. Clamps were filled rapidly and rolled using a tractor mounted buck rake. Once both clamps were filled, they were covered with a double layer of plastic silage sheet and weighed down with car tyres. Clamps were opened on 21st October 2002.

4.2.1.2 Grass silage

Grass, from a predominantly perennial ryegrass (*Lolium perenne* L.) sward, was mown on 29th May 2002, wilted for 24 h and precision chopped (3 cm TCL) using a trailed forage harvester (3625, John Deere, Nottingham, UK). Precision chopped grass was ensiled without an additive in a plastic lined, concrete walled, roofed clamp covered with a single sheet of oxygen impermeable silage sheet and a tarpaulin, and weighed down with gravel bags (Silo stop, Inocen, Spain).

4.2.1.3 Fermented whole-crop wheat (WCW)

A commercially managed stand of winter wheat, *cv.* Equinox, was harvested at a target DM of 440 g kg^{-1} FM, and precision chopped (3 cm TCL) using a self propelled forage harvester (Class Jaguar 800, Bury St. Edmunds, UK), leaving a stubble height of approximately 22 cm. A fermentation enhancer (Wholecrop gold, Biotal, Cardiff, UK), was applied by the forage harvester at the rate of 4l t^{-1} FM. The precision chopped, additive treated material was ensiled in a plastic lined, concrete walled, roofed clamp and covered with a double layer of plastic silage sheet and weighed down with tyres.

4.2.2 Animals and experimental diets

4.2.2.1 In sacco degradation characteristics

Four mature, ruminally fistulated, Suffolk cross wethers, with a mean live-weight of 95 kg (s.d.=1.8), were fed the same basal diet at the rate of 1.05x maintenance (AFRC, 1993), and kept under the same conditions, as described in Section 3.2.3.4. Sheep were fed at 08:00 and 20:00 h daily. Each meal consisted of 520 g FM good quality field hay and 220 g FM concentrate (70:30 DM basis; composition described in Section 3.2.3.4.).

4.2.2.2 Metabolism experiment and lamb growth experiment

Fifty-two Suffolk cross wether lambs with a mean live-weight of 30 kg (s.d.=2.9), that were grazing grass, were selected for the study. The lambs were weighed, conditioned scored, ear tagged, wormed (6 ml per head of a 40 ml l^{-1} ricobendazole oral drench, Crown Veterinary Pharmaceuticals, Hertfordshire, UK) and injected, sub-cutaneously, with a 2 ml dose of Ovivac P-Plus (Intervet, UK) to protect against clostridia and pasteurella. The six heaviest lambs were selected for use in the metabolism study (Section 4.2.4). The remaining 48 lambs were split into two groups, with the 24 heavier lambs taken off pasture and housed immediately and the 24 lighter lambs were left out at pasture and supplemented with good quality grass hay for a further week prior to being reweighed and housed.

The forage portion of the experimental diets was solely grass silage (GS) or a mixture, 1:1 DM basis, of GS and either high tannin pea silage or low tannin pea silage (HT and LT respectively). The experimental concentrate (LP) was formulated to provide sufficient metabolisable energy and protein for a 35 kg wether lamb consuming 400 g concentrate d^{-1} to grow at 150 g d^{-1} when offered GS (384 g kg^{-1} FM, 11.1 MJ kg^{-1} DM, 112 g CP kg^{-1} DM by NIRS) *ad libitum* (AFRC, 1993). The LP concentrate consisted of 985.5 g kg^{-1} ground barley and 17.5 g kg^{-1} feed grade urea. The LP concentrate was formed into pellets

measuring approximately 6 by 20 mm (diameter x length) using a pelleting machine. Each forage mix was fed with either 400 g d^{-1} LP or 400 g d^{-1} LP with an additional 200 g d^{-1} pelleted soya-bean meal (HP), resulting in six dietary treatments:

GS *ad libitum* & 400 g d^{-1} LP	GS+LP
GS *ad libitum* & 400 g d^{-1} LP & 200 g d^{-1} soya	GS+HP
HT *ad libitum* & 400 g d^{-1} LP	HT+LP
HT *ad libitum* & 400 g d^{-1} LP & 200 g d^{-1} soya	HT+HP
LT *ad libitum* & 400 g d^{-1} LP	LT+LP
LT *ad libitum* & 400 g d^{-1} LP & 200 g d^{-1} soya	LT+HP

4.2.3 Experiment 2a - *In sacco* degradability

Core samples of the two pea silages and the WCW were taken from five random locations across the top of each clamp, at an approximate depth of 40 cm. Whole-crop wheat was included in the determination of degradability characteristics in order to facilitate the formulation of balanced diets for dairy cows in Experiment 3. Grass silage samples were taken from five random locations across the clamp, at approximately 25 cm behind the exposed face. The samples were mixed by hand, and stored at -20°C. Approximately 8 g FM, of each individual silage, was accurately weighed into a pre-labelled, precision woven, mono filamentous polyester fibre bag, with internal dimensions of 23 x 9 cm and an aperture size of 43 x 43 µm. The bags had rounded bottoms, to prevent lodging of the samples, and were sealed by passing the neck of the bag through a brass curtain ring (18 mm diameter), folding the neck of the bag back against itself, and securing it with a coloured elastic band which identified the treatment. Once the bags were made up they were stored at +4°C prior to use, for a maximum period of 2 d.

Six bags were connected to a stainless steel clip, which was attached to the cannular cap by a 30 cm length of nylon cord. Each forage was incubated in the rumen for 2, 4, 8, 16, 24, 36, 48 and 72 h using a complete exchange method (Paine *et al.*, 1982), as detailed in Appendix 1. Each forage was incubated in each sheep in triplicate at all time points in order to ensure sufficient residue for analysis. Post incubation, the bags were placed in a bucket of cold water to remove rumen debris, prior to being washed, on a cold cycle, for 40 min in a commercial washing machine (Electra, program D). In addition, zero hour time points were washed through the cold wash cycle. The bags and contents were dried to a constant weight at 60°C, and the contents bulked, within each sheep at each time point. The empty bags were re-washed, dried and examined for tears prior to further use.

4.2.4 Experiment 2b - Metabolism experiment

Six lambs were housed indoors, individually, in slatted floor pens (3 m^2), with free access to fresh clean drinking water, under continuous lighting, in a temperature maintained room (15°C) for the duration of the experiment. During the first week, lambs were offered grass silage *ad libitum* and received 100 g LP at 16:00 h on the second day, 100 g LP for the following three meals, and 200 g LP for the subsequent meals. Lambs were sheared at the end of the first week. At the beginning of the second week they were randomly allocated to one of the six experimental diets in an incomplete changeover design (Table 4.1), with each experimental period lasting 21 d. Each period consisted of 14 d adaptation followed by 7 d sampling.

Table 4.1 Diet allocation of six Suffolk cross wether lambs fed forage diets *ad libitum*, based on, solely grass silage (GS), or a 1:1 mixture, on a DM basis, of GS and either high tannin pea silage (HT) or low tannin pea silage (LT), supplemented with either 400 g d^{-1} low protein concentrate (LP) or 400 g d^{-1} of LP with an additional 200 g d^{-1} high protein soya-bean meal (HP).

	Sheep					
Period	A	B	C	D	E	F
1	GS+LP	HT+LP	GS+HP	HT+HP	LT+LP	LT+HP
2	HT+LP	LT+HP	HT+HP	GS+HP	GS+LP	LT+LP
3	LT+HP	HT+HP	LT+LP	GS+LP	GS+HP	HT+LP
4	LT+LP	GS+LP	HT+LP	LT+HP	HT+HP	GS+HP

Fresh forage was taken each morning from each clamp and mixed by hand, where appropriate, with grass silage on a 1:1 DM basis and offered at 1.10 x the previous recorded intake, determined by recording refusals every Tuesday and Thursday morning. In order to maintain the desired DM ratio, silage samples were dried to a constant weight at 100°C on Monday and Thursday of each week and dietary adjustments made accordingly.

During the first 7 d of each experimental period, lambs were housed individually in slatted floor pens and were fed silage at 08:30 h with concentrate being fed in two equal meals at 08:00 h and 16:00 h by adding a bucket to each pen that was removed when empty. On day 8, the faecal collection harnesses were fitted and the lambs transferred into metabolism crates. The faecal collection bag was left unzipped in order to allow faecal material to escape. The forage mixes were placed into the hoppers on the front of the crate at 08:30 h daily and the concentrate was fed in equal portions at 08:00 h and 16:00 h by placing it at the bottom of the hoppers. Fresh water was available *ad libitum*. On day 14, refusals were recorded prior to the morning feed, the lambs removed from the crates and weighed, and the faecal collection bags were lined with a plastic bag and sealed and the lambs returned to the metabolism crate. Urine was collected by gravity into a collection tray and preserved with 200 ml of 2 M sulphuric acid. Prior to the morning feed, faecal matter was removed from the collection bag and a new liner installed. Faecal material was mixed by hand and weighed. A proportion of the faecal matter (0.1 kg kg^{-1}) was stored frozen (-20°C) prior to subsequent analysis. Total urine output was determined by weighing the collected urine,

after filtering it through glass wool. The urine was diluted to 4 kg by adding deionised water, and two sub-samples of 100 ml were stored at -20°C prior to subsequent analysis.

Silage and concentrate samples were taken daily and stored at -20°C prior to subsequent analysis. Refusals were recorded every day during the sampling period, prior to morning feeding, and daily feed intake adjusted to 1.10x previous days recorded intake. At the end of each period, the harnesses were removed, washed, and the lambs reweighed and returned to the slatted floor pens and the diet changed.

4.2.5 Experiment 2c - Lamb growth

Each of the 24 lambs from each batch was blocked by live-weight and randomly allocated to one of the six experimental diets (4.2.2.2). Lambs in batch one started receiving experimental diets 7 d before those in batch two. The lambs were housed individually, in slatted floor pens (3 m^2), with *ad libitum* access to clean drinking water in an open fronted barn. For the first two days, lambs were introduced to silage by feeding grass silage as the sole forage component of their diet.

Forage mixes were made daily, as described in Section 4.2.4 and fed at 08:30 h. Refusals were recorded each Tuesday and Thursday and the lambs fed at 1.10x previous recorded intake. Concentrate was fed in two equal meals, at 08:00 h and 16:00 h daily by placing a bucket into each pen that was removed when empty. During the first week of housing, concentrate was increased in stages. Lambs received no concentrate on the first day, 100 g at 16:00 h on the second day, 100 g for the following three meals, 200 g for the next three meals and finally either 200 or 300 g for the subsequent meal times, depending on dietary regime. There were no concentrate refusals at any time.

Silage and concentrate samples were taken weekly and stored at -20°C prior to subsequent analysis. Lambs were weighed weekly, on a Tuesday morning 2 h post morning feed, in

portable calf scales (IAE, Leek, Staffordshire, UK) that were calibrated prior to use, using standard weights. Each batch of lambs was blood sampled, by jugular venepuncture, 10 ml into lithium heparin vacutainers (Bioscience Int. Plc., Bridgend, UK), at 11:00 h (3 h post morning feed) on the Tuesday of weeks 0, 2, 4 and 6 of their respective experimental period. Blood samples were centrifuged at 2800 xg for 10 min, and the plasma transferred into micro centrifuge tubes (1.5 ml) and stored frozen at -80°C to await further analysis. After 8 weeks, from date of commencement, the lambs were weighed and removed from their pens at 14:00 h and group housed overnight in straw bedded pen, with access to fresh clean drinking water *ad libitum*. The following morning the lambs were taken to a commercial slaughterhouse. Following electrical stunning, lambs were blood sampled at the point of exsanguination, with a sub-sample of blood being collected into two lithium heparin vacutainers (Bioscience Int. Plc., Bridgend, UK). Prior to hygiene inspection, carcasses were split longitudinally using a band saw and the right hand side numbered for identification purposes. Hot carcass weight (both halves) was determined within 1 h of slaughter using commercially calibrated scales. Carcasses were cooled overnight (+4°C) and reweighed (both halves) the following morning in order to determine cold carcass weight, the right hand side was wrapped in plastic and stored at +4°C prior to analysis.

4.2.6 Analysis

4.2.6.1 Experiment 2a - In sacco degradability

Frozen silage samples were defrosted slowly at +4°C, and analysed for DM and N as described in Chapter 2. In addition, samples were analysed for acid detergent fibre (ADF), in quadruplicate, by the method of Goering and Van Soest (1979), using Fibretec apparatus (FOSS, Warrington, UK). Prior to ashing, the residues from two replicates of each silage were combined and analysed for acid detergent insoluble nitrogen (ADIN) using the N method described in Chapter 2.

Water soluble and small particle losses for each of the forages were determined by an adaptation of the method of Weisbjerg *et al.* (1990). Approximately 10 g of each defrosted, non-ruminally incubated silage was accurately weighed, in triplicate, into a 500 ml glass beaker, to which 200 ml de-ionised water was added. Each beaker was swirled 10 times every 15 min for 1 h. The solution was then filtered through a dried, pre-weighed Whatman 541 filter paper (15 cm diameter, ashless, pore size 16 μm) under vacuum, and the beaker rinsed three times with 50 ml of de-ionised water. The filter paper and retainate were dried at 60°C for 48 h, prior to being reweighed. The retainate was analysed for N as described in Chapter 2. Water soluble loss (*WSL*) was calculated as follows

$$WSL = \frac{(X_{in} - X_{retained})}{X_{in}} \qquad \text{Equation 4.1}$$

where X is either g of DM or N. The bulked bag residues (~1.5 g DM) were milled in a coffee grinder, and analysed for N as described in Chapter 2.

4.2.6.2 Experiment 2b - Metabolism experiment

Silage and concentrate samples were defrosted slowly and bulked within each period and analysed for DM, ash, N, NDF, starch and WSC by the methods described in Chapter 2. In

addition, silage samples were also analysed for pH, NH_3-N, tannin and VFA as described in Chapter 2.

Estimated ME was calculated using Equation 3.1 for the pea silages. Grass silage ME was calculated using the equation of Givens *et al.* (1989), where

Grass silage estimated ME = 5.45 + 0.0085[NCD] Equation 4.2

Concentrate ME was estimated using the equation of MAFF (1993), where

Concentrate ME = 0.014[NCDG] + 0.025[EE] Equation 4.3

Faecal samples were bulked for each lamb within each period and analysed for DM, ash, N and NDF by the methods described in Chapter 2. In addition, silage, concentrate and faecal samples (exactly 0.500 g) were analysed for gross energy by adiabatic bomb calorimetry (AOAC, 2000). Urine samples were bulked for each lamb within each period by combining 10 ml from each days collection. Exactly 5 ml of urine was used for the determination of N by the method described in Chapter 2. One batch of bulked urine was refrozen and sent away for determination of purine derivatives, by the methods described by Chen and Gomes (1995), at the Rowett Research Institute, Aberdeen, UK.

Daily purine absorption was calculated using the iterative Newton-Raphson equation described by Chen *et al.* (1990), where Y is the sum of all the purine derivatives (mmol d^{-1}) excreted, and X_1 = Y/0.84 (Equation 4.4) and $W^{0.75}$ is the metabolic live-weight of the sheep.

$$X_{(n+1)} = X_n - \frac{0.84X_n + 0.150W^{0.75} \times e^{-0.25X_n} - Y}{0.84X_n - 0.038W^{0.75} \times e^{-0.25X_n}} \quad \text{Equation 4.5}$$

Microbial N yield (g d^{-1}) was calculated by multiplying the result of X_6 (the 6th iterative result) by 0.727 (Equation 4.6).

Diet digestible energy (DE) was calculated from total gross energy (GE) digestibility, using total GE intake and GE output recorded over the 7 d sampling period, and corrected for dry matter intake.

$$\text{Diet DE}_{(\text{MJ kg}^{-1}\text{ DM})} = \frac{\left(\frac{\text{GE}_{in} - \text{GE}_{out}}{\text{GE}_{in}}\right) \text{x GE}_{diet}}{\text{Total dry matter intake}_{(kg)}} \qquad \text{Equation 4.7}$$

Diet metabolisable energy (ME) was calculated by multiplying the diet DE by 0.81 (Equation 4.8, ARC, 1980). The ME content of the concentrate component was calculated using standard values reported in AFRC (1993), where the ME of urea, ground barley and hi-pro soya-bean meal were taken as 0, 13.3 and 13.3 MJ kg^{-1} DM respectively, resulting in an ME content of 13.0 and 12.9 MJ kg^{-1} DM for the LP and HP concentrate respectively. The ME of the forage mix was determined using Equation 4.9.

$$\text{ME forage mix}_{(\text{MJ kg}^{-1}\text{ DM})} = \frac{\text{Diet ME}_{(\text{MJ kg}^{-1}\text{ DM})} - (\text{ME}_{conc} \text{ x CDMI}_{(kg)})}{\text{forage dry matter intake}_{(kg)}} \qquad \text{Equation 4.9}$$

Where conc = concentrate and CDMI = concentrate dry matter intake.

Individual pea silage ME was calculated by subtracting half the value of the grass silage ME, determined in the same period with the same concentrate, from the $ME_{forage\ mix}$ and doubling the result, assuming no interaction between the forages and the concentrates.

4.2.6.3 Experiment 2c - Lamb growth

Half carcasses were split transversely between the penultimate and caudal rib using a saw. The kidney was removed from the carcass and the fat around it was separated and weighed. Hind leg circumference was measured using a plastic coated tape measure. The transverse section length and transverse section width of the eye muscle of the penultimate rib was measured using a steel rule and subcutaneous fat depth measured to the nearest millimetre using a set of metal callipers (Figure 4.1).

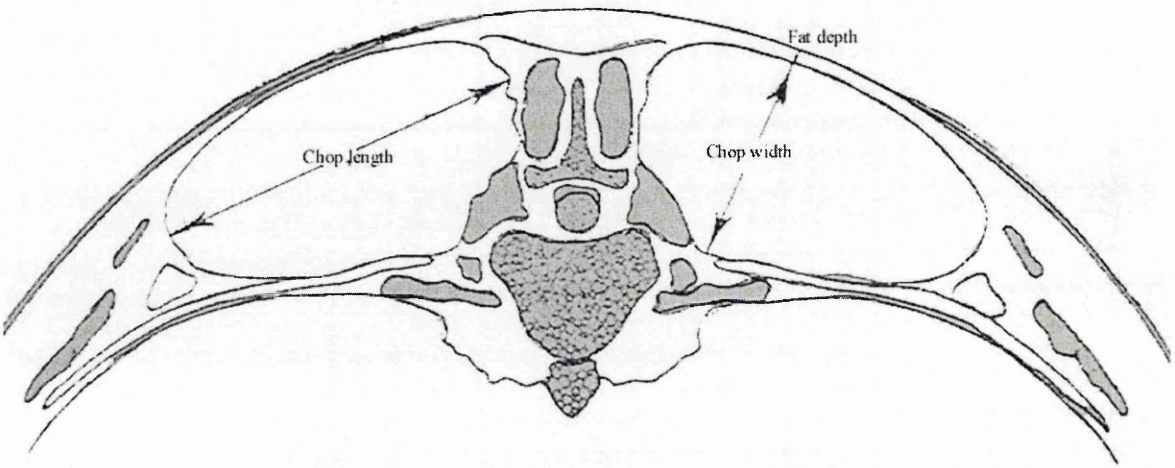


Figure 4.1 Dimensional analysis of the eye muscle, adapted from Brown and Williams (1979).

The eye muscle area was traced, and its area determined, after scanning into a computer, using DT scan (Delta Scan, version 2.06). All carcass measurements were made on the day after slaughter. Killing out proportion was calculated by dividing cold carcass weight by slaughter weight.

Silage and concentrate samples were defrosted slowly and bulked into three week periods and analysed as described in 4.2.5.2 with the exception of GE. Plasma samples were defrosted slowly at room temperature and analysed for urea and β hydroxybutyrate (βHB) by the methods described in Chapter 2.

4.2.7 Calculations and statistical analysis

4.2.7.1 Crop yield

Crop yield was calculated, on a t ha^{-1} basis, for each quadrat. Yields were subjected to analysis using the ANOVA procedure of Genstat, with crop type used as the treatment factor.

4.2.7.2 Experiment 2a - In sacco degradability

Data for both DM and N was fitted, without zero hour values, to the first order model of Ørskov and McDonald (1979), $P = a + b \times 1 - \exp^{-ct}$ (Equation 4.10), using Sigmaplot (version 8.0, SPSS Inc.), for each feed within each sheep, where a is the immediately soluble fraction, b the potentially soluble fraction and c the rate at which b is degraded in the rumen. Effective degradability (ED) was calculated using the equation of Ørskov and McDonald (1979), where a, b and c are calculated using equation 4.10 and r is the rumen outflow rate, either 0.05 or 0.08 h^{-1}, and t is time set to infinity (9999999 h).

$$ED = a + \left(\frac{b \times c}{c + r}\right) \times 1 - \exp^{-(c+r)t} \qquad \text{Equation 4.11}$$

Effective degradability (CED), corrected for water soluble losses, was calculated using the equation of Weisbjerg *et al.* (1990), where WSL is calculated using equation 4.1, a from equation 4.10 and ED from equation 4.11 (all expressed as a proportion of 1)

$$CED = WSL + \frac{1 - WSL}{1 - a} \times (ED - a) \qquad \text{Equation 4.12}$$

All curve parameters and degradabilites were analysed using the ANOVA procedure of Genstat, with silage type being the treatment and sheep used as a blocking factor.

Dietary parameters were calculated using the following equations from AFRC (1992)

Quickly degradable protein (QDP) = a x [CP] Equation 4.13

Slowly degradable protein (SDP) = {(b x c)/(c + r)} x [CP] Equation 4.14

Effective rumen degradable protein (ERDP) = 0.8 [SDP] + [QDP] Equation 4.15

Undegradable dietary protein (UDP) = [CP] – ([QDP]+[SDP]) Equation 4.16

Digestible undegraded protein (DUP) = 0.9 {[UDP]-(6.25[ADIN])} Equation 4.17

4.2.7.3 Experiment 2b - Metabolism experiment

Mean chemical composition, of both the silages and concentrates, was determined using the ANOVA procedure of Genstat, with period being used as the blocking factor. Intake, digestibility, purine derivative output, N balance and energy balance were analysed as an incomplete latin square using a 3 x 2 (forage mix x concentrate) factorial design using the ANOVA procedure of Genstat, with sheep nested by period (period/sheep) as the blocking factor. Calculated forage mix ME was analysed using the ANOVA procedure of Genstat, with silage type as the treatment factor, with sheep nested by period (period/sheep) as the blocking factor.

4.2.7.4 Experiment 2c - Lamb growth

Mean chemical composition, of both the silages and concentrates, was determined as described in 4.2.6.3. Average daily live-weight gain (ADLWG) was calculated by means of linear regression using Microsoft Excel. Feed conversion efficiencies (FCE; kg gain kg^{-1} DMI) were calculated by dividing total weight gain by total DM intake. Data were analysed using a 3 x 2 (forage mix x concentrate) factorial randomised block design using the ANOVA procedure of Genstat. Initial weight was used as a covariate for ADLWG,

slaughter weight, and for all carcass traits measured. Blood data from week 0 were used as a covariate for weeks 2, 4, 6 and 8. Data were analysed from 47 lambs, as one lamb was removed from the experiment for health reasons that were not associated with dietary treatment.

4.3 Results

4.3.1 Crop yield

The mean yields for FM, DM and CP for the two pea varieties are presented in Table 4.2.

There was no statistical difference in FM, DM or CP yield between the pea varieties.

Table 4.2 Fresh matter (FM), dry matter (DM) and crude protein (CP) yields of high and low tannin peas harvested for ensilage (t ha^{-1})

	High tannin	Low tannin	s.e.d.	*P*
FM	47.4	42.8	5.20	0.393
DM	9.70	9.19	1.111	0.657
CP	1.94	1.73	0.219	0.357

4.3.2. Silage composition

The mean chemical composition of the grass silage and whole-crop pea silages used in experiments 2a, 2b and 2c are presented in Table 4.3.

Table 4.3 Mean chemical composition of the grass silage and whole-crop pea silages used in experiments 2a, 2b and 2c (all g kg^{-1} DM, unless otherwise stated).

	Grass silage	High tannin pea silage	Low tannin pea silage	s.e.d.	*P*
DM (g kg^{-1} FM)	404^{b}	309^{a}	323^{a}	17.0	0.003
OM	928^{b}	900^{a}	927^{b}	3.1	<0.001
pH	3.9	4.1	4.0	0.25	0.150
CP	118^{a}	200^{b}	188^{b}	7.7	<0.001
NH_3-N (g kg^{-1} TN)	101	135	140	17.0	0.124
WSC	19.9	18.1	18.1	6.51	0.953
Starch	28.0^{a}	72.0^{b}	64.8^{b}	7.54	0.013
NDF	498^{b}	281^{a}	262^{a}	10.5	<0.001
ADF	300^{b}	216^{a}	209^{a}	4.3	<0.001
EE	28.3^{b}	20.5^{a}	15.8^{a}	2.77	0.011
GE (MJ kg^{-1} DM)	17.7^{b}	17.3^{a}	17.4^{a}	0.08	0.005
Est ME† (MJ kg^{-1} DM)	11.8^{a}	12.1^{b}	12.6^{c}	0.10	<0.001
Tannin (TAE‡)	n.d.	93.1^{b}	47.2^{a}	1.12	<0.001
VFAs					
Acetate	21.7^{a}	39.0^{a}	64.6^{b}	9.59	0.012
Propionate	1.15	0.80	0.54	0.348	0.281
Butyrate	1.34	2.19	1.03	1.239	0.647
Valerate	0.00	0.23	0.49	0.176	0.083

† Calculated from laboratory analysis using Equation 3.1 (peas) or 4.2 (grass), ‡ tannic acid equivalents (g kg^{-1} DM), n.d. not determined. Means in the same row, not sharing a common superscript letter differ ($P<0.050$)

The grass silage had a higher DM than either the high tannin or low tannin pea silage. The OM content of the high tannin pea silage was lower than that of either the grass silage or low tannin pea silage. There was no difference between silages in terms of pH with a mean value of 4.0. The CP of the grass silage was relatively low, with a value of 118 g CP kg^{-1} DM, whereas the pea silages had similar values of 200 and 188 g CP kg^{-1} DM for high tannin and low tannin respectively. Ammonia nitrogen concentrations were moderate and not statistically different, with a mean value of 124 g kg^{-1} TN. There was no difference between silages in terms of residual WSC content, with a mean content of 18.7 g kg^{-1} DM. The pea silages had a higher starch content, a mean of 68.4, compared to 28.0 g kg^{-1} DM for the grass silage. The pea silages had a lower NDF content, a mean of 272 compared to 498 g kg^{-1} DM for the grass silage. The grass silage had a higher ADF concentration, a mean of 300 g kg^{-1} DM compared to a mean value of 213 g kg^{-1} DM for the pea silages. The EE content was highest in the grass silage, with no difference being observed between either of the pea silages. Measured GE was highest in the grass silage with no difference between the pea silages. Estimated ME and was highest in the low tannin pea silage and lowest in the grass silage. The high tannin pea silage had a higher tannin content compared to the low tannin pea silage. The low tannin pea silage had a higher acetate content compared to either the grass or high tannin pea silage. There was no difference between silages in terms of propionate, butyrate and valerate concentrations, with only low values (<5 g kg^{-1} DM) being recorded.

The WCW used in experiment 2a had a DM, OM, pH, CP, NH_3-N, WSC, starch, NDF, ADF and EE content of 392, 967, 3.9, 86, 170, 14.8, 225, 340, 185 and 10.1 g kg^{-1} respectively, tannin, ME, GE and VFAs were not determined.

4.3.3 Experiment 2a - *In sacco* degradability

4.3.3.1 Dry matter degradation characteristics

The immediately soluble DM fraction (a) of the silages was highest in the WCW and lowest in the grass silage, with intermediate values for the pea silages (Table 4.4 and Figure 4.2).

Table 4.4 *In sacco* DM degradability coefficients, water soluble loss (WSL), effective degradability (ED) and corrected effective degradability (CED) of grass silage (GS), high tannin whole-crop pea silage (HT) and low tannin whole-crop pea silage (LT) and whole-crop wheat (WCW)

	GS	HT	LT	WCW	s.e.d.	*P*
a	0.328^{a}	0.415^{b}	0.446^{c}	0.488^{d}	0.0121	<0.001
b	0.510^{c}	0.405^{b}	0.408^{b}	0.330^{a}	0.0121	<0.001
a+b	0.779^{a}	0.820^{b}	0.854^{c}	0.818^{b}	0.0116	0.001
c	0.052^{a}	0.090^{b}	0.086^{b}	0.040^{a}	0.0070	<0.001
WSL	0.353^{a}	0.484^{b}	0.472^{b}	0.263^{a}	0.0043	0.002
ED 0.05 h^{-1}	0.528^{a}	0.676^{c}	0.702^{d}	0.632^{b}	0.0055	<0.001
CED 0.05 h^{-1}	0.582^{b}	0.714^{c}	0.717^{c}	0.470^{a}	0.0098	<0.001
ED 0.08 h^{-1}	0.469^{a}	0.630^{c}	0.658^{d}	0.596^{b}	0.0060	<0.001
CED 0.08 h^{-1}	0.529^{b}	0.673^{c}	0.673^{c}	0.418^{a}	0.0098	<0.001

Means in rows not sharing common superscript letter differ, $P<0.050$

The high tannin and low tannin pea silages had similar potentially degradable fractions (b) and similar rates of degradation (c). The grass silage had the lowest potentially degradable fraction and slowest rate of degradation (Figure 4.2 and Table 4.4). The grass silage had the lowest total degradable fraction and the low tannin pea silage the highest (Table 4.4). The proportion of the immediately soluble fraction, that was water soluble, was lowest in the grass silage and highest in the high tannin pea silage (Table 4.4). Effective degradability, at outflow rates of 0.05 and 0.08 h^{-1}, was lowest in the grass silage and highest in the low tannin pea silage (Table 4.4). The corrected effective degradability, calculated at outflow rate of 0.05 and 0.08 h^{-1}, was lowest in the grass silage, but no difference was observed between the low and high tannin pea silages, with an intermediate value for the WCW (Table 4.4).

The coefficient of determination (r^2) values for DM were 0.948, 0.936, 0.944 and 0.933 for grass silage, high tannin pea silage, low tannin pea silage and WCW respectively.

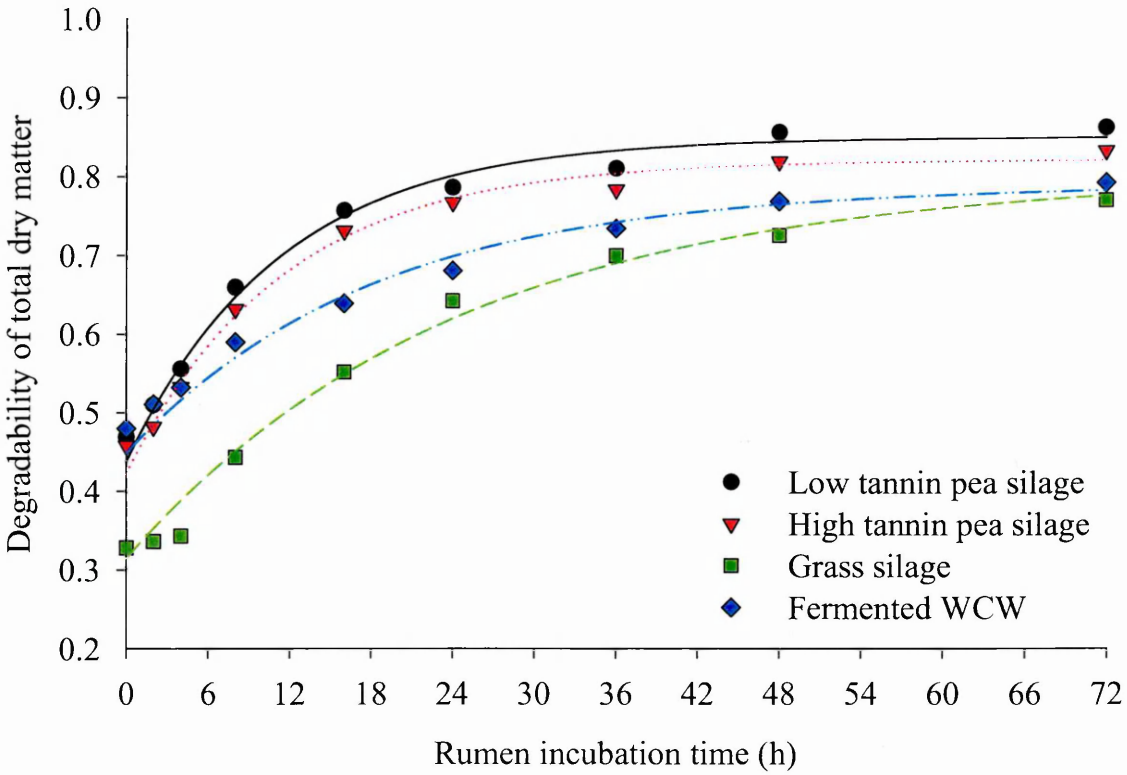


Figure 4.2 In sacco DM degradation of whole-crop silages, symbols actual values, and lines fitted values

4.3.3.2 Nitrogen degradation characteristics

The grass silage had the lowest immediately soluble N fraction, with the highest being seen in the low tannin pea silage (Table 4.5 and Figure 4.3). The largest potentially soluble fraction was seen in the grass silage and the lowest in the WCW (Table 4.5). The fastest rate of degradation was seen in the WCW and the lowest in the grass silage (Table 4.5). Total potential N degradation (a+b) was highest in the low tannin pea silage and lowest in the grass silage (Table 4.5 and Figure 4.3). The water soluble loss was similar between the grass silage and high tannin pea silage, with the WCW having the highest water soluble loss (Table 4.5).

Table 4.5 *In sacco* N degradability coefficients, water soluble loss (WSL), effective degradability (ED) and corrected effective degradability (CED) of grass silage (GS), high tannin whole-crop pea silage (HT) and low tannin whole-crop pea silage (LT) and whole-crop wheat (WCW)

	GS	HT	LT	WCW	s.e.d.	*P*
a	0.659^{a}	0.744^{b}	0.785^{c}	0.772^{c}	0.0107	<0.001
b	0.215^{c}	0.194^{c}	0.166^{b}	0.104^{a}	0.0092	<0.001
a+b	0.874^{a}	0.938^{b}	0.951^{b}	0.876^{a}	0.0064	<0.001
c	0.057^{a}	0.107^{a}	0.255^{ab}	0.362^{b}	0.0937	0.036
WSL	0.487^{a}	0.482^{a}	0.587^{b}	0.634^{b}	0.0183	0.003
ED 0.05 h^{-1}	0.771^{a}	0.875^{c}	0.919^{d}	0.857^{b}	0.0058	<0.001
CED 0.05 h^{-1}	0.654^{a}	0.746^{b}	0.843^{c}	0.769^{b}	0.0018	<0.001
ED 0.08 h^{-1}	0.746^{a}	0.854^{b}	0.905^{c}	0.850^{b}	0.0069	<0.001
CED 0.08 h^{-1}	0.618^{a}	0.704^{b}	0.817^{d}	0.758^{c}	0.0020	<0.001

Means in rows not sharing common superscript letter differ, $P<0.050$

Effective degradability, at rumen outflow rates of 0.05 and 0.08 h^{-1}, was lowest in the grass silage and highest in the low tannin pea silage (Table 4.5). The corrected effective degradability was also lowest in the grass silage and highest in the low tannin pea silage at both rumen outflow rates (Table 4.5).

The N degradability characteristics are shown graphically in Figure 4.3.

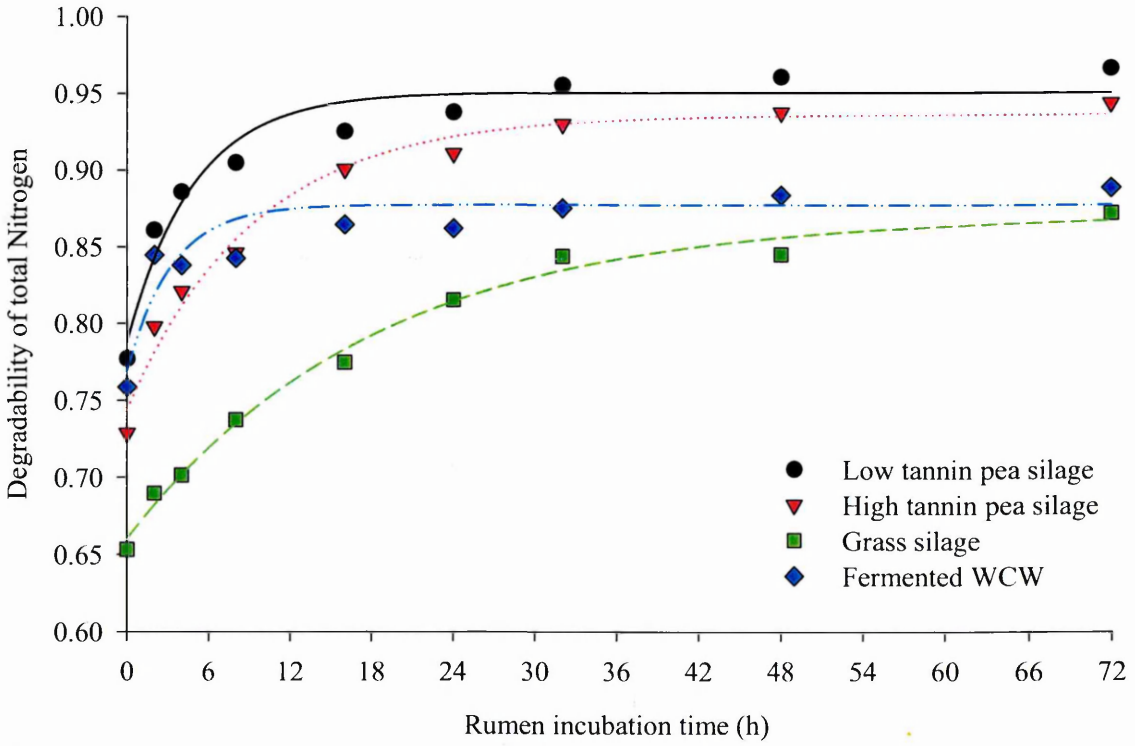


Figure 4.3 In sacco N degradation of whole-crop silages, symbols mean values, and lines fitted values

The r^2 values for N degradability were 0.929, 0.956, 0.859 and 0.724 for grass silage, high tannin pea silage, low tannin pea silage and WCW respectively.

Protein degradation characteristics are presented in Table 4.6. The low protein silages, i.e. the grass and WCW, had similar ERDP levels at both rumen outflow rates, but a greater proportion of the grass silage crude protein was degradable post ruminally. The pea silages had similar, high levels, of ERDP, but the high tannin peas silage contained twice the level of post ruminally digestible protein (DUP) compared to the low tannin pea silage at both rumen outflow rates.

Table 4.6 Metabolisable protein degradation parameters, of core samples of grass silage (GS), high tannin pea silage (HT), low tannin pea silage (LT) and whole-crop wheat silage (WCW) determined in situ (all g kg^{-1} DM)

	GS	HT	LT	WCW
CP	99	196	182	86
Acid detergent insoluble nitrogen	0.67	1.22	0.91	0.39
Quickly degradable protein	65	146	143	66
Slowly degradable protein 0.05 h^{-1}	11.3	25.9	25.3	7.9
Slowly degradable protein 0.08^{-1}	8.7	21.7	23.0	7.3
Undegradable dietary protein 0.05 h^{-1}	22.5	24.3	13.9	11.7
Undegradable dietary protein 0.08 h^{-1}	24.9	28.4	16.9	12.2
Effective rumen degradable protein 0.05 h^{-1}	64	143	140	61
Effective rumen degradable protein 0.08 h^{-1}	61	138	137	60
Digestible undegraded protein 0.05 h^{-1}	16.5	15.0	7.4	8.4
Digestible undegraded protein 0.08 h^{-1}	18.7	18.7	9.5	8.8

4.3.4. Experiment 2b – Metabolism experiment

4.3.4.1 Concentrate composition

The chemical composition of the low and high protein concentrates fed during experiments 2b and 2c are presented in Table 4.7. The LP concentrate had a higher DM, OM and EE compared to the HP concentrate. The estimated ME and measured GE of the HP concentrate was higher than that of the LP concentrate. The HP concentrate had an extra 123 g CP kg^{-1} DM and 17.1 g WSC kg^{-1} DM compared to the LP concentrate. There was

no difference in terms of NDF. The LP concentrate contained 164 g starch kg^{-1} DM more than the HP concentrate.

Table 4.7 Mean chemical composition of the low and high protein concentrates fed during the lamb growth and metabolism experiment (all g kg^{-1} DM, unless otherwise stated).

	Low protein concentrate	High protein concentrate
DM (g kg^{-1} FM)	848	831
OM	978	959
CP	169	292
WSC	20.3	37.4
Starch	513	349
NDF	164	162
EE	17.0	13.8
GE (MJ kg^{-1} DM)	18.5	18.9
Est ME† (MJ kg^{-1} DM)	13.2	13.4

† Calculated from laboratory analysis using Equation 4.3

4.3.4.2 Intake and apparent digestibility

Average daily forage intakes and total DMI were unaffected by dietary treatment (Table 4.8). There was a trend (P=0.056) suggesting that the apparent whole tract DM digestibility of the lambs fed the LT forage was higher than those fed the GS forage. Apparent whole tract DM digestibility was highest (P<0.001) in the lambs fed the HP concentrate with a mean value of 0.782 kg kg^{-1} compared to 0.744 kg kg^{-1} for the LP concentrate (s.e.d.=0.0068). The apparent whole tract digestibility of OM was highest (P<0.050) in lambs fed the LT forage mix, 0.771 kg kg^{-1}, and lowest in the lambs fed the GS forage, 0.748 kg kg^{-1}, with an intermediate value of 0.759 kg kg^{-1} for those fed the HT forage mix (s.e.d.=0.0082). Lambs fed the HP concentrate had a higher (P<0.001) apparent whole tract OM digestibility compared to those fed the LP concentrate (0.778 and 0.741 kg kg^{-1} respectively, s.e.d=0.0067).

Apparent whole tract NDF digestibility was highest (P<0.050) in the lambs fed the GS forage, with no difference being observed between the HT and LT forage mixes (0.665,

0.606 and 0.611 kg kg^{-1} respectively, s.e.d.=0.0142). Apparent whole tract NDF digestibility was highest ($P<0.001$) in lambs fed the HP concentrate compared to those fed the LP concentrate (0.658 and 0.598 kg kg^{-1} respectively, s.e.d.=0.0116). Apparent GE digestibility was highest ($P<0.050$) in the lambs fed the LT forage and lowest for those fed GS with an intermediate value for those fed HT (0.756, 0.726 and 0.742 kg kg^{-1} respectively, s.e.d.=0.0082). Lambs consuming the HP concentrate had a higher ($P<0.001$) apparent GE digestibility than those consuming the LP concentrate, with values of 0.723 and 0.719 kg kg^{-1} respectively (s.e.d.=0.0067). There were no forage x concentrate interactions.

Table 4.8 Voluntary intakes and apparent organic matter (OM) and neutral detergent fibre (NDF) digestibility of Suffolk cross wether lambs offered forage diets *ad libitum*, based on grass silage (GS), or a 1:1 mixture, on a DM basis, of GS and either high tannin pea silage (HT) or low tannin pea silage (LT), supplemented with either 400 g d^{-1} low protein concentrate (LP) or, 400 g d^{-1} of LP with an additional 200 g d^{-1} high protein soya-bean meal (HP)

	Diet						Significance			
	GS		HT		LT		Forage Mix		Concentrate	
	LP	HP	LP	HP	LP	HP	s.e.d.	*P*	s.e.d.	*P*
Silage intake (kg FM d^{-1})	1.79	1.97	2.49	2.04	2.40	1.95	0.247	0.283	0.201	0.246
Forage DMI (kg DM d^{-1})	0.71	0.79	0.91	0.74	0.88	0.71	0.091	0.725	0.075	0.273
Forage DMI (g kg^{-1} W0.75 d^{-1})	41.3	44.9	51.4	42.9	50.1	41.9	4.66	0.679	3.81	0.268
Total DMI (kg DM d^{-1})	1.05	1.29	1.25	1.24	1.22	1.21	0.091	0.722	0.075	0.342
Apparent DM digestibility	0.739	0.767	0.737	0.786	0.756	0.793	0.0083	0.056	0.0068	<0.001
Apparent OM digestibility	0.735	0.760	0.733	0.785	0.755	0.788	0.0082	0.036	0.0067	<0.001
Apparent NDF digestibility	0.645	0.685	0.565	0.648	0.583	0.640	0.0142	0.001	0.0116	<0.001
Apparent GE digestibility	0.709	0.743	0.711	0.772	0.738	0.773	0.0176	0.009	0.0143	<0.001

There were no forage mix x concentrate interactions

4.3.4.3 Digestible energy and estimated metabolisable energy content

Table 4.9 presents the DE and ME of the whole diet and the ME content of the forage mixes and individual forages. Diet DE was highest ($P<0.050$) in lambs offered the LT forage and lowest for those offered GS, with an intermediate value for those offered HT (13.6, 13.2 and 13.5 MJ kg^{-1} DM respectively, s.e.d.=0.16). The inclusion of soya-bean meal, in lambs fed HP, resulted in a higher ($P<0.001$) diet DE of 13.9 MJ kg^{-1} DM compared to 12.9 MJ kg^{-1} DM (s.e.d.=0.16) for those fed the LP concentrate. The diet ME content was highest ($P<0.050$) in lambs offered LT and lowest in those offered GS, with an intermediate value for those offered HT (11.0, 10.7 and 10.8 MJ kg^{-1} DM respectively, s.e.d.=0.13). The diet ME was highest ($P<0.001$) in lambs fed the HP concentrate compared to those fed the LP concentrate (11.3 and 10.4 MJ kg^{-1} DM respectively, s.e.d.=0.11).

The ME of the LT forage mix was higher ($P<0.050$) than that of the GS forage, with an intermediate value for the HT forage (9.87, 9.27 and 9.56 MJ kg^{-1} DM respectively, s.e.d.=0.168). The ME of the forage mixes was affected by the inclusion of soya-bean meal, with a mean value of 9.81 MJ kg^{-1} DM compared to 9.33 MJ kg^{-1} DM when soya-bean meal was not included. The ME of the low tannin pea silage was higher ($P<0.050$) than that of the grass silage, with an intermediate value for the high tannin pea silage (Table 4.9).

Table 4.9 Mean diet DE, diet ME, forage mix ME and individual forage ME determined using Suffolk cross wether lambs offered forage diets *ad libitum*, based on grass silage (GS), or a 1:1 mixture, on a DM basis, of GS and either high tannin pea silage (HT) or low tannin pea silage (LT), supplemented with either 400 g d^{-1} low protein concentrate (LP) or, 400 g d^{-1} of LP with an additional 200 g d^{-1} high protein soya-bean meal (HP).

	Diet						Significance			
	GS		HT		LT		Forage Mix		Concentrate	
	LP	HP	LP	HP	LP	HP	s.e.d.	*P*	s.e.d.	*P*
Diet DE (MJ kg^{-1} DM)	12.8	13.6	12.7	14.0	13.2	14.1	0.16	0.045	0.13	<0.001
Diet ME (MJ kg^{-1} DM)	10.3	11.0	10.3	11.4	10.7	11.4	0.13	0.046	0.11	<0.001
Forage mix ME (MJ kg^{-1} DM)	9.1	9.5	9.2	10.0	9.8	10.0	0.17	0.010	0.14	0.003
Forage ME (MJ kg^{-1} DM)	9.27		9.86		10.48		0.370	0.014	-	-

There were no forage mix x concentrate interactions

4.3.4.4 Nitrogen balance and microbial protein synthesis

Table 4.10 shows the N utilisation and microbial protein synthesis of lambs offered the six experimental diets. Total N intake of lambs offered the HT and LT forage mixes was higher ($P<0.050$) than those offered the GS forage (36.2, 34.6 and 29.4 g N d^{-1} respectively, s.e.d.=2.13). Lambs consuming the HP concentrate had a higher ($P<0.001$) N intake compared to those consuming the LP concentrate (38.9 and 27.9 g N d^{-1} respectively, s.e.d.=1.74). Total N output was higher ($P<0.050$) in lambs fed the HT or the LT forage mix compared to the GS (24.6, 22.9 and 18.9 g N d^{-1} respectively, s.e.d.=1.09). Feeding the HP concentrate resulted in an increased ($P<0.001$) N output compared to those fed the LP concentrate (25.6 and 18.7 g N d^{-1} respectively, s.e.d.=0.89). There were no treatment effects on faecal N output, with a mean value of 8.1 g N d^{-1}. Total urinary N output was lowest ($P<0.001$) from lambs fed the GS forage compared to either the HT or LT forage mix (11.0, 15.8 and 15.4 g N d^{-1} respectively, s.e.d.=0.94). Feeding the HP concentrate resulted in an increased ($P<0.001$) urinary N output compared to the LP concentrate (17.4 and 10.8 g N d^{-1} respectively, s.e.d.=0.77). Nitrogen balance was unaffected by forage mix. However, lambs fed the HP concentrate had a higher ($P<0.010$) N retention compared to those fed the LP concentrate (13.4 and 9.2 g N d^{-1} respectively, s.e.d.=1.37).

When faecal, urinary and retained N were expressed as a proportion of total N intake, there was no difference between any of the treatments in terms of N retained, with a mean proportion of 0.333 (Figure 4.4). The proportion of N excreted in the faeces was highest ($P<0.050$) in lambs fed the GS forage, and lowest in those fed the LT forage mix (Figure 4.4). Lambs fed the LP concentrate had a higher ($P<0.001$) faecal N output compared to those fed the HP concentrate (0.290 and 0.207 respectively, s.e.d.=0.0090). The proportion of N excreted in the urine was affected by concentrate type, with the HP having a higher ($P<0.050$) value compared to the LP concentrate (0.453 and 0.384 respectively,

s.e.d.=0.0308). There was no significant effect of dietary treatment on the efficiency of N utilisation, with a mean value of 0.332 g g^{-1}. Apparent whole tract digestibility of N was highest (P<0.050) in lambs fed the LT forage mix and lowest in those fed the GS forage, with an intermediate value for those fed the HT forage mix (0.779, 0.720 and 0.753 respectively, s.e.d.=0.0107). Lambs fed diets containing the HP concentrate had a higher (P<0.001) apparent whole tract digestibility of N compared to those fed diets containing the LP concentrate (0.792 and 0.709 respectively, s.e.d.=0.0088).

Purine derivative (PD) excretion was unaffected by any of the dietary treatments, but there was a tendency (P=0.077) for those lambs fed the HT forage mix and the LP concentrate to have elevated levels of all PD measured (Table 4.11). Microbial protein synthesis was unaffected by any of the dietary treatments, with a mean value of 14.8 g N d^{-1}. There were no forage x concentrate interactions.

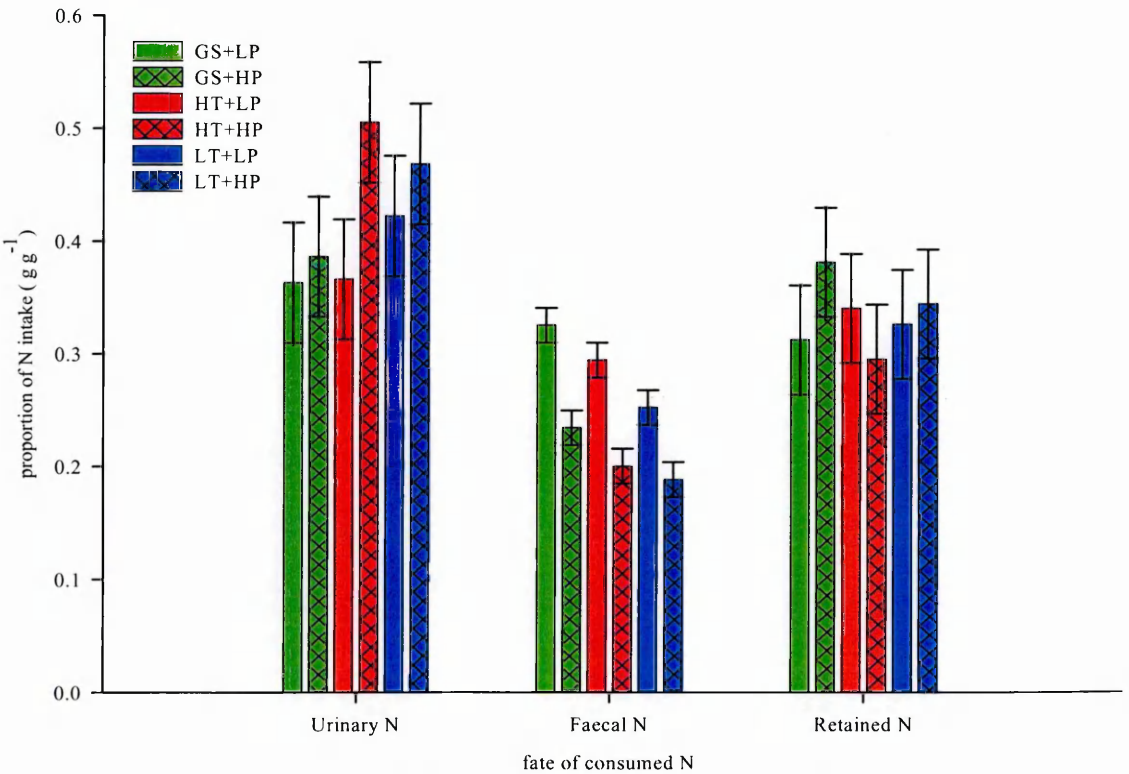


Figure 4.4 Metabolic fate of dietary nitrogen (N), as a proportion of total N intake of wether lambs offered forage diets *ad libitum*, based on grass silage (GS), or a 1:1 mixture, on a DM basis, of GS and either high tannin pea silage (HT) or low tannin pea silage (LT), supplemented with either 400 g d^{-1} low protein concentrate (LP) or, 400 g d^{-1} of LP with an additional 200 g d^{-1} high protein soya-bean meal (HP)

Table 4.10 Nitrogen (N) utilisation and microbial protein synthesis (MPS) of Suffolk cross wether lambs offered forage diets *ad libitum*, based on grass silage (GS), or a 1:1 mixture, on a DM basis, of GS and either high tannin pea silage (HT) or low tannin pea silage (LT), supplemented with either 400 g d^{-1} low protein concentrate (LP) or, 400 g d^{-1} of LP with an additional 200 g d^{-1} high protein soya-bean meal (HP). All g d^{-1} unless otherwise stated.

	Diet						Significance			
	GS		HT		LT		Forage Mix		Concentrate	
	LP	HP	LP	HP	LP	HP	s.e.d.	*P*	s.e.d.	*P*
Total N intake	22.1	36.7	31.6	40.8	30.0	39.3	2.13	0.016	1.74	<0.001
Silage	12.9	14.2	22.4	18.3	20.8	16.8	2.17	0.018	1.77	0.223
Concentrate	9.2	22.5	9.2	22.5	9.2	22.5	0.13	1.000	0.11	<0.001
Total N output	15.2	22.7	20.8	28.3	20.1	25.7	1.09	<0.001	0.89	<0.001
Faeces	7.16	8.68	9.19	8.27	7.53	7.44	0.704	0.230	0.575	0.768
Urine	8.0	14.0	11.6	20.0	12.6	18.3	0.94	<0.001	0.78	<0.001
N balance	6.9	14.0	10.8	12.5	9.9	13.6	1.68	0.721	1.37	0.008
Apparent N digestibility (g g^{-1})	0.675	0.765	0.708	0.798	0.745	0.813	0.0107	<0.001	0.0088	<0.001
Efficiency N utilisation (g g^{-1})	0.310	0.382	0.337	0.295	0.325	0.345	0.0351	0.695	0.0286	0.569
Purine derivatives (PD)										
allantoin (mmol d^{-1})	7.6	9.8	10.2	8.5	8.0	8.5	0.76	0.388	0.62	0.605
uric acid (mmol d^{-1})	0.87	1.04	1.19	0.80	1.10	1.12	0.114	0.427	0.093	0.490
X + H† (mmol d^{-1})	6.74	7.15	7.77	7.58	7.51	7.52	0.471	0.294	0.385	0.841
total PD (mmol d^{-1})	15.2	18.0	19.1	16.9	16.7	17.1	1.01	0.357	0.83	0.687
MPS (gN d^{-1})	13.1	15.5	16.5	14.6	14.4	14.8	0.88	0.356	0.72	0.689

† sum of (X)anthine and (H)ypoxanthine. There were no forage mix x concentrate interactions

4.3.5 Experiment 2c - Lamb growth

4.3.5.1 Lamb dry matter intakes and performance

There was no difference between the mean starting weight of the lambs in each block, with a mean weight of 30.0 kg (s.d.=3.06, Table 4.13). The mean voluntary intake, FCE and average daily liveweight gain (ADLWG) of the lambs are presented in Table 4.11. Lambs offered the HT and LT diets consumed more silage than those on the GS diets (2.48, 2.46 and 2.19 kg FM d^{-1} respectively, s.e.d.=0.123, P=0.036). However, when silage intakes were compared on a dry matter basis or corrected for metabolic live weight ($W^{0.75}$), no differences were observed between forages (P=0.814 and 0.949 respectively). Silage intakes were also unaffected by concentrate type. Total dry matter intake was unaffected by forage type, but the lambs fed the LP concentrate consumed 180 g DM d^{-1} less than those fed the HP concentrate (P<0.001). The mean weekly live weight of the lambs is presented graphically in Figure 4.5.

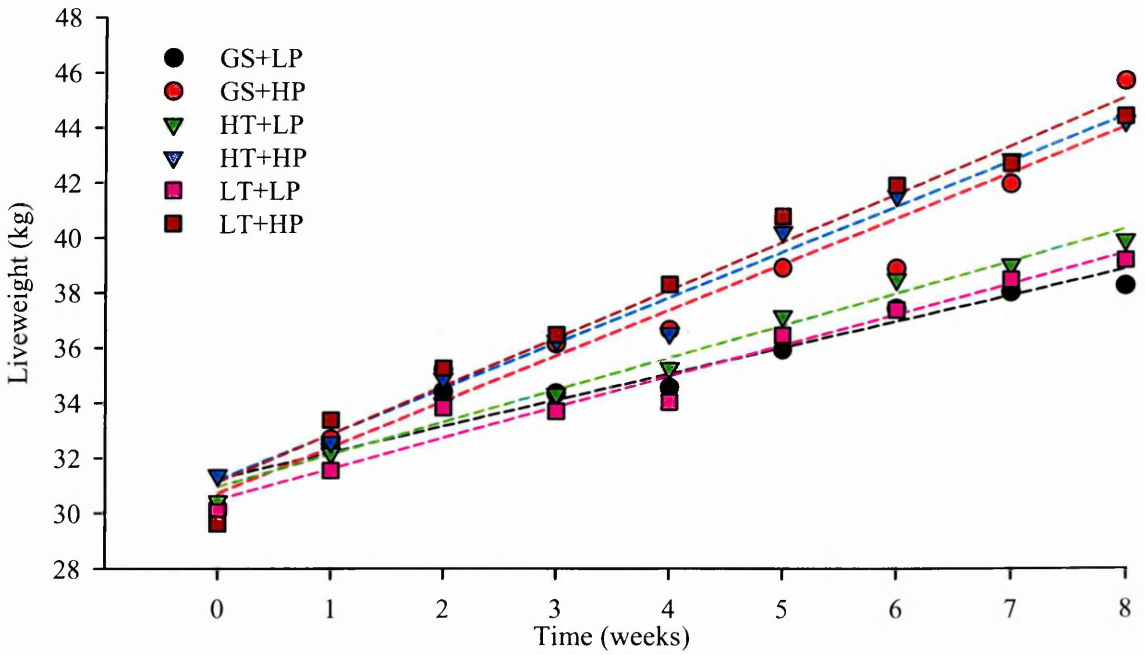


Figure 4.5 Mean liveweight (symbols) of wether lambs fed forage diets *ad libitum*, based on, solely grass silage (GS), or a 1:1 mixture, on a DM basis, of GS and either high tannin pea silage (HT) or low tannin pea silage (LT), supplemented with either 400 g d^{-1} low protein concentrate (LP) or, 400 g d^{-1} of LP with an additional 200 g d^{-1} high protein soya-bean meal (HP)

Lambs fed the HP concentrate grew, on average, at 235 g d^{-1} compared to 158 g d^{-1} for those fed the LP concentrate (s.e.d.= 11.9, $P<0.001$). Lambs offered the GS forage grew 20.9 g d^{-1} slower than those offered the HT and LT forage mixes. The lambs fed the GS had a lower FCE compared to those fed the HT and LT forage mixes (0.132, 0.154 and 0.162 kg gain kg^{-1} DMI, s.e.d.=0.0101, $P=0.016$). Lambs fed the HP concentrate had a higher FCE than those fed the LP concentrate (0.168 and 0.131 kg gain kg^{-1} DMI, s.e.d.=0.0168, $P<0.001$).

Table 4.11 Mean voluntary intake, food conversion efficiencies (FCE) and average daily liveweight gain (ADLWG), of Suffolk wether cross lambs offered forage diets *ad libitum*, based on grass silage (GS), or a 1:1 mixture, on a DM basis, of GS and either high tannin pea silage (HT) or low tannin pea silage (LT), supplemented with either 400 g d^{-1} low protein concentrate (LP) or, 400 g d^{-1} of LP with an additional 200 g d^{-1} high protein soya-bean meal (HP)

	Diet						Significance			
	GS		HT		LT		Forage Mix		Concentrate	
	LP	HP	LP	HP	LP	HP	s.e.d.	*P*	s.e.d.	*P*
Forage intake (kg FM d^{-1})	2.19	2.18	2.43	2.54	2.46	2.47	0.123	0.036	0.204	0.747
Forage DMI (kg d^{-1})	0.87	0.87	0.85	0.90	0.90	0.90	0.046	0.814	0.037	0.576
Forage DMI (g kg^{-1} $W^{0.75}$ d^{-1})	59.9	56.7	58.4	58.3	60.8	57.2	2.44	0.949	1.99	0.262
Total DMI (kg d^{-1})	1.21	1.37	1.18	1.40	1.23	1.40	0.046	0.800	0.038	<0.001
FCE (kg gain kg^{-1} DMI)	0.112	0.152	0.140	0.169	0.141	0.184	0.0101	0.016	0.0083	<0.001
ADLWG (g d^{-1})	135	208	167	244	172	252	14.8	0.018	11.9	<0.001

There were no forage x concentrate interactions

4.3.5.2 Blood metabolites

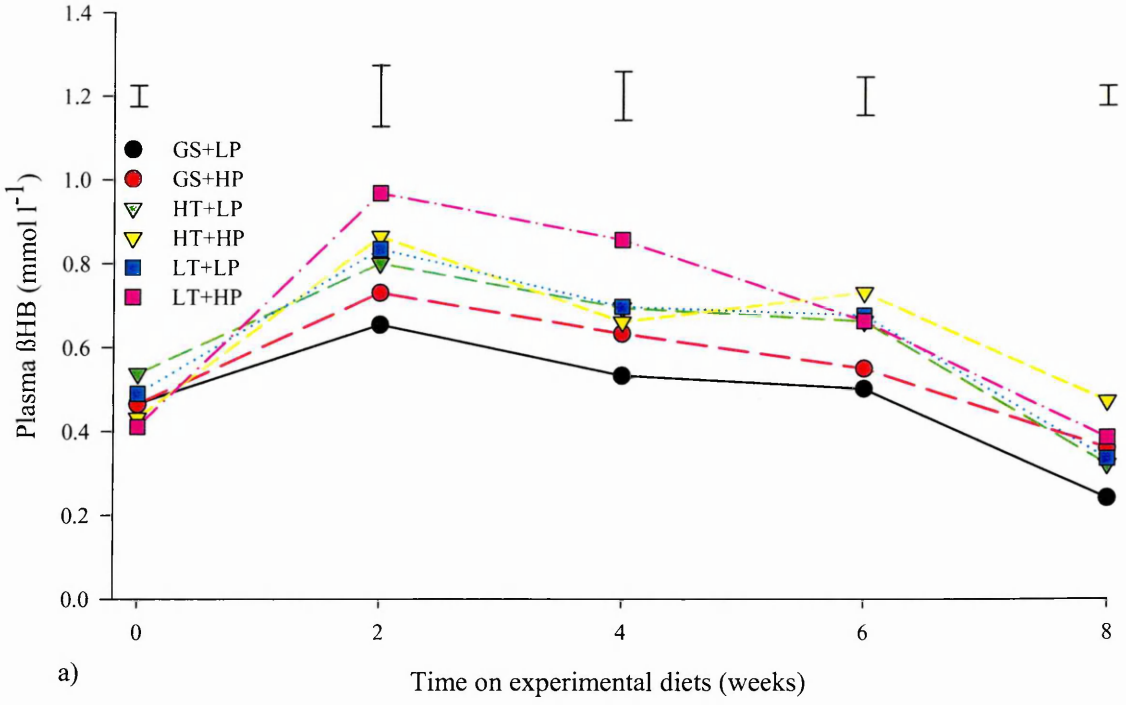
Mean plasma βHB and urea values are presented in Table 4.12. Lambs fed GS had the lowest βHB, with a value of 0.525 mmol l^{-1}, whilst the lambs fed the HT or LT forage mixes had mean βHB concentrations of 0.650 and 0.676 mmol l^{-1} respectively (s.e.d.=0.0460, P=0.002). There was a trend (P=0.051) for lambs fed the HP concentrate to have a higher mean βHB compared to those fed the LP concentrate (0.656 and 0.579 mmol l^{-1}, s.e.d.=0.0397). Plasma βHB concentration, measured fortnightly, is displayed in Figure 4.6a. There was a significant effect of concentrate type at week 0 (P=0.041). There were no differences in mean βHB during weeks 2 and 4. However, during week 6, lambs offered the HT and LT forage mixes had higher plasma βHB concentrations compared to those offered GS (0.697, 0.669 and 0.526 mmol l^{-1} respectively, s.e.d.=0.0635, P=0.023). At the point of slaughter (week 8), lambs fed the GS diet had the lowest plasma βHB concentration at 0.301 mmol l^{-1}, and lambs fed the HT had the highest concentration at 0.937 mmol l^{-1}, with an intermediate value of 0.360 mmol l^{-1} for the lambs fed the LT forage mix (s.e.d.=0.0329, P=0.016). The mean plasma βHB concentration, at the point of slaughter, of the lambs fed the HP concentrate was higher (P<0.001) than those fed the LP concentrate (0.406 and 0.299 mmol l^{-1} respectively, s.e.d.=0.0284).

Mean plasma urea nitrogen (PUN) was highest in lambs fed diets containing the HT forage mix and lowest in lambs offered GS, with an intermediate value for those offered LT (8.45, 7.35 and 8.21 mmol l^{-1} respectively, s.e.d.= 0.247, P<0.001). Lambs consuming diets supplemented with the HP concentrate had higher (P<0.001) PUN compared to those fed the LP concentrate (9.53 and 6.48 mmol l^{-1} respectively, s.e.d.=0.197). The 'forage mix x concentrate' interaction is presented graphically in Figure 4.6b. There was no difference in PUN between any of the dietary treatments at week 0. There was no difference between PUN of lambs fed any forage mix with the HP concentrate at weeks 2, 4, 6 and 8, which was in contrast to those fed diets with the LP concentrate. Lambs fed the GS+LP diet had

lower (P<0.050) PUN concentrations than those fed either the HT+LP or LT+LP diets during weeks 2, 4 and 6.

Table 4.12 Mean plasma, beta-hydroxybutyrate (βHB), and urea nitrogen of Suffolk cross wether lambs, offered forage diets *ad libitum*, based on grass silage (GS), or a 1:1 mixture, on a DM basis, of GS and either high tannin pea silage (HT) or low tannin pea silage (LT), supplemented with either 400 g d^{-1} low protein concentrate (LP) or, 400 g d^{-1} of LP with an additional 200 g d^{-1} high protein soya-bean meal (HP).

	Diet						Significance					
	GS		HT		LT		Forage mix (F)		Concentrate (C)		FxC	
	LP	HP	LP	HP	LP	HP	s.e.d.	*P*	s.e.d.	*P*	s.e.d.	*P*
βHB (mmol l^{-1})	0.482	0.568	0.619	0.682	0.635	0.717	0.0460	0.002	0.0397	0.051	0.0661	0.966
Urea N (mmol l^{-1})	5.15	9.54	7.42	9.48	6.87	9.56	0.247	<0.001	0.197	<0.001	0.348	<0.001



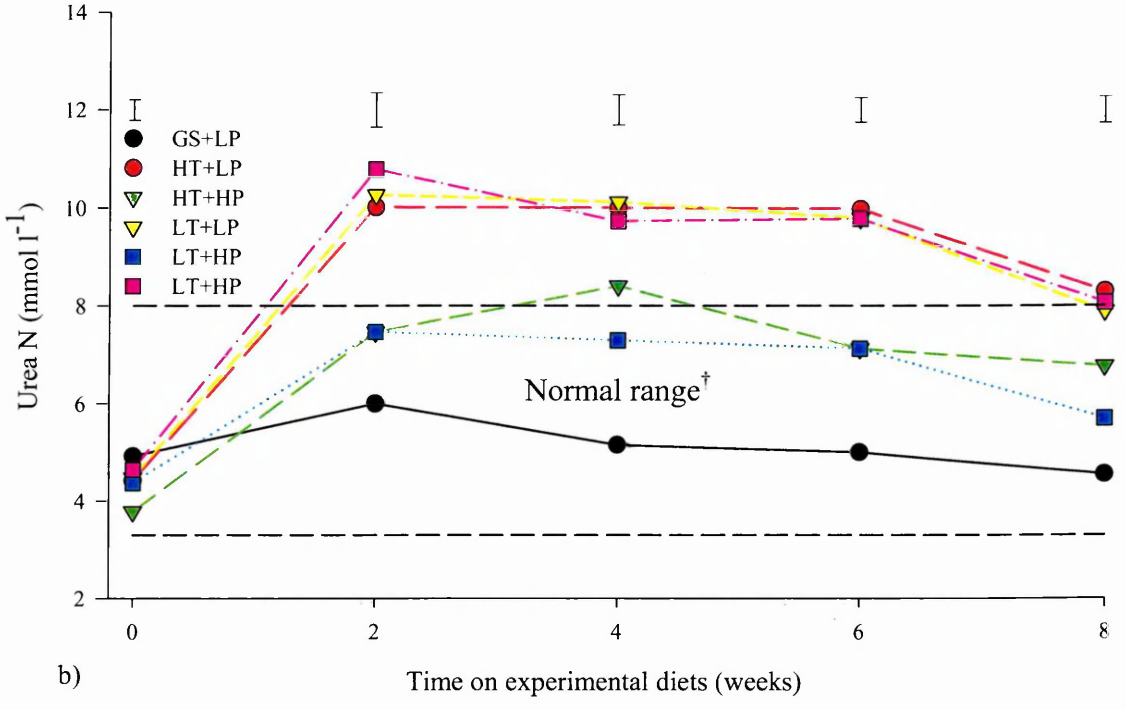


Figure 4.6 Mean βHB (a) and plasma urea nitrogen (b) of lambs fed forage diets *ad libitum*, based on, solely grass silage (GS), or a 1:1 mixture, on a DM basis, of GS and either high tannin pea silage (HT) or low tannin pea silage (LT), supplemented with either 400 g d^{-1} low protein concentrate (LP) or, 400 g d^{-1} of LP with an additional 200 g d^{-1} high protein soya-bean meal (HP) †Normal range as defined by Radostits *et al.* (2003)

4.3.5.3 Carcass characteristics

The mean carcass weights and carcass traits are presented in Table 4.13 Forage mixture did not affect slaughter weight, but lambs fed diets containing the HP concentrate had a higher (P<0.001) slaughter weight (43.8 kg) compared to those fed the LP concentrate (39.0 kg; s.e.d.=0.77). Lambs that had been fed the LT forage mix had a higher (P<0.050) hot carcass weight compared to those fed the GS forage, with an intermediate value for those fed the HT forage mix (19.9, 18.5 and 19.5 kg respectively, s.e.d.=0.56). The lambs that had been fed the HP concentrate had a higher (P<0.001) hot carcass weight compared to those fed the LP concentrate, with values of 20.3 and 18.3 kg respectively (s.e.d.=0.45). There was a tendency (P=0.076) for lambs consuming the LT forage mix to have a higher cold carcass weight than those fed the GS forage mix. Lambs fed the HP concentrate had a higher (P<0.001) cold carcass weight compared to those fed the LP concentrate, with values of 20.0 and 18.0 kg respectively (s.e.d.=0.43).

There was no dietary effect on killing out proportion with a mean value of 0.466 kg kg^{-1}. Hind leg circumference was unaffected by forage mix, but lambs fed the HP concentrate had an increased (P<0.001) hind leg circumference compared to those fed the LP concentrate (42.6 and 40.9 cm respectively, s.e.d.=0.33). There was no effect of dietary treatment on kidney knob and channel fat weight, with a mean value of 157 g. Lambs fed diets containing the LT forage mix had longer (P<0.050) chop lengths compared to those fed the GS and HT forage mixes, with values of 6.49, 6.15 and 6.09 cm respectively (s.e.d.=0.129). Lambs fed the HP concentrate had a greater (P<0.010) chop length compared to those fed the LP concentrate, with values of 6.39 and 6.09 cm respectively (s.e.d.=0.104). Chop width was unaffected by forage mix. However, lambs fed the HP concentrate had a greater chop width of 3.00 cm compared to 2.79 cm for those fed the LP concentrate (s.e.d.=0.085). Lambs fed the GS forage had the lowest (P<0.050) subcutaneous fat depth of 1.54 mm compared to 2.66 and 3.07 mm for the lambs fed the

HT and LT forage mixes respectively (s.e.d.=0.388). Subcutaneous fat depth was unaffected by concentrate type. There was no effect of forage mix on the eye muscle area. However, there was a tendency (P=0.076) for the lambs fed the HP concentrate to have an increased eye muscle area compared to those fed the LP concentrate (1454 and 1368 mm^2 respectively, s.e.d.=46.6).

Table 4.13 Mean weights and carcass traits of Suffolk cross wether lambs, offered forage diets *ad libitum*, based on grass silage (GS), or a 1:1 mixture, on a DM basis, of GS and either high tannin pea silage (HT) or low tannin pea silage (LT), supplemented with either 400 g d^{-1} low protein concentrate (LP) or, 400 g d^{-1} of LP with an additional 200 g d^{-1} high protein soya-bean meal (HP)

	Diet						Significance			
	GS		HT		LT		Forage Mix		Concentrate	
	LP	HP	LP	HP	LP	HP	s.e.d.	*P*	s.e.d.	*P*
Initial weight (kg)	30.0	30.2	30.3	30.1	29.8	29.6	0.38	0.426	0.31	0.873
Slaughter Weight (kg)	38.2	42.6	39.5	44.2	39.3	44.6	0.96	0.225	0.77	<0.001
Hot carcass weight (kg)	17.6	19.4	18.6	20.4	18.6	21.2	0.56	0.045	0.45	<0.001
Cold carcass weight (kg)	17.5	19.0	18.3	20.1	18.2	20.9	0.54	0.072	0.43	<0.001
Killing out proportion (kg kg^{-1})	0.460	0.455	0.471	0.461	0.471	0.476	0.0132	0.511	0.0106	0.770
Hind leg circumference (cm)	40.8	42.1	40.7	42.9	41.1	42.8	0.42	0.431	0.332	<0.001
Kidney fat (g)	151	150	153	160	146	179	17.5	0.781	14.1	0.371
Chop length (cm)	6.15	6.15	5.83	6.35	6.30	6.68	0.129	0.009	0.181	0.007
Chop width (cm)	2.65	2.92	2.90	3.05	2.81	3.05	0.106	0.181	0.085	0.015
Subcutaneous fat depth (mm)	1.75	1.32	2.22	3.10	2.67	3.47	0.388	0.001	0.313	0.190
Eye muscle area (mm^2)	1297	1412	1375	1464	1432	1486	57.8	0.202	46.6	0.076

There were no forage x concentrate interactions

4.4 Discussion

4.4.1 Crop yield

The yields of DM achieved in this experiment for the high tannin and low tannin whole-crop pea silages (9.70 and 9.19 t DM ha^{-1} respectively) were greater than those of 6.02 and 5.93 t DM ha^{-1} respectively, determined in Experiment 1, using the same pea varieties and soil conditions, and are comparable to those discussed in Section 3.4.1.1. The DM yields of the whole-crop pea silage are higher than that of 6.82 t DM (Chadwick, 2004) for a two cut grass silage receiving 220 kg N ha^{-1}. This difference between the two experiments may be explained by the difference in growth stage at harvest, with the plant being 5 d more mature, but it is more likely that this 3 t difference is a reflection of inaccuracies in yield determination in both experiments. However, work by Fraser *et al.* (2001) found that there was no difference between DM yields of peas harvested at growth stages 204, 205 and 207 (Knott, 1987), corresponding to 10, 12 and 14 weeks maturity. Work by Potts (1980) concluded that DM yield varied greatly between seasons, with different patterns of growth being observed between wet and dry periods and years. The mean CP yield (1.83 t ha^{-1}) of the whole-crop pea silages was higher than that of 0.95 t ha^{-1} determined in Experiment 1. The CP yield achieved in this experiment was higher than that of 0.97 t ha^{-1} determined by Fraser *et al.* (2001).

4.4.2 Silage composition

The chemical analysis of the grass silage indicates that it is of low nutritive quality, with a high DM, low CP content and high NDF content, reflective of the late stage of maturity at harvest. The pea silages produced in this experiment had a higher DM of 316 g kg^{-1} FM, a higher CP of 194 g kg^{-1} DM, an increased level of NH_3-N (138 g kg^{-1} TN), a decreased WSC and starch content of 18.1 and 68.4 g kg^{-1} DM respectively compared to those produced after wilting and treatment with a fermentation enhancer in Experiment 1. The

high tannin pea variety contained 1.97 fold more tannin than the low tannin pea, a figure comparable to the 1.8 fold difference observed in Experiment 1. The tannin concentration had increased from a mean of 22.0 g TAE kg^{-1} DM pre-ensiling in Chapter 3 to a mean of 70.2 g TAE kg^{-1} DM post ensiling in the current study. This difference may reflect a change in the distribution of tannins from fibre-bound to free tannins, a possible effect of the ensiling process, or a limitation of the acid butanol method for tannin analysis (Makkar *et al.*, 1999). The relatively high levels of acetate, determined in the whole-crop pea silages (51.8 g kg^{-1} DM), were higher than those observed by Fraser *et al.* (2001, 11.7 g kg^{-1} DM). This difference may be an indication of a fermentation dominated by *L. buchneri*, a hetero-fermentative lactic acid bacteria which was present in the additive applied. Hetero-fermentative bacteria ferment 1 mole of fructose into 1 mole of lactate and 1 mole of acetate, or 1 mole of glucose into 1 mole of lactate and 1 mole of ethanol, in contrast to homo-fermentative lactic acid bacteria which ferment 1 mole of glucose or fructose into 2 moles of lactate (McDonald, 1973). The low pH coupled with a low level of butyrate and moderate NH_3-N concentration in the pea silages is indicative of little secondary fermentation by clostridia (Gibson, 1965).

4.4.3 Experiment 2a - *In sacco* degradability

4.4.3.1 In sacco degradation characteristics

Contamination of samples by microbial N has been shown, by Varvikko and Lindberg (1985) to be affected by initial CP concentration of the samples. Estimates of N losses from feedstuffs containing a low level of CP, e.g. straw, resulted in an underestimate of N loss of 1285% after 24 h rumen incubation, but feedstuffs containing a high level of CP, e.g. rapeseed meal, were only underestimated by 0.7% after rumen incubation for 24 h. The samples used in this experiment ranged from 86 to 196 g CP kg^{-1} DM, so an

underestimate of degradation may have been observed in the grass silage and the WCW, but was unlikely to have had a major effect in either of the pea silages.

The processing of the samples prior to ruminal incubation within the polyester bags can also cause estimates of DM and N to be either under or overestimated, and hence the sample should mimic how the feed is presented to the animal (Huntington and Givens, 1995). The recommended *in situ* method of AFRC (1992) suggested that silages, fresh or frozen, should be chopped by hand to a length of approximately 1 cm. In the present study, silages were presented in the bags as they would have been fed to an animal i.e. not chopped or ground. This led to a reduced surface area to volume ratio, and a less homogenous substrate in comparison to a dried ground sample, since they had not undergone any form of mechanical mastication.

Work by Emanuele and Staples (1988) has shown that when forages were dried and ground through a 2 mm sieve, approximately half of the ground material passed through a 0.5 mm sieve, so comparisons between dried, ground samples and fresh, unprocessed samples may not necessarily be a true representation of the degradation characteristics. The estimation of the water soluble loss, and hence the small particle losses, as described by Weisbjerg *et al.* (1990), were made under the assumption that the particle loss is representative of the sample, which is not always the case as discussed by Huntington and Givens (1995). There are differences between the way that grass and legume particles fragment in the rumen, with grasses breaking up into long thin shards, that may pass through the holes in the polyester bags, and legumes into short, broad, almost cubical particles that are less likely to pass out of the polyester bag (Emanuele and Staples, 1988). Therefore, the estimation of the immediately soluble fraction (a) may have been overestimated in both the grass silage and the WCW.

Mustafa *et al.* (2000; 2002), determined the *in sacco* degradation characteristics of 4 whole-crop pea silages that had been dried and ground through a 2 mm screen. The average immediately soluble fraction of 0.773 (s.d.=0.058) and potentially degradable fraction of 0.200 (s.d.=0.083) were comparable to the means of 0.765 and 0.180 respectively, obtained in this study. However, the rate term (c) of 0.104 (s.d.=0.031) was more comparable to the mean of 0.107 for the high tannin pea silage determined in this study.

In this study, the high tannin pea silage had a lower immediately soluble fraction and a lower potential total degradability (a+b) compared to the low tannin variety, therefore providing more DUP. Although the potential degradable fraction (b) was highest in the high tannin pea silage in comparison to the low tannin pea silage, the mean rate term was less than half that of the low tannin pea silage. However, there was no statistical difference between the high and low tannin varieties. Messman (1996) examined the *in situ* degradability of forage legumes varying in tannin content (undefined mixtures of hydrolysable and condensed tannins), and concluded that higher levels of tannin resulted in no difference in the immediately soluble fraction (a), but a numerically higher proportion of protein remained after 12 h rumen incubation compared to the lower tannin varieties. This was in contrast to a study by Robbins *et al.* (1987a), who found that significant levels of tannins reduced protein solubility in deer (*Odocoleus hemionus*) and a study by Julier *et al.* (2002), who found that the addition of purified condensed tannin to lucerne reduced protein solubility by a proportion of 0.57. The addition of chestnut tannin (a hydrolysable tannin) to ground pea seeds, in an experiment carried out by Poncet and Remond (2002), was found to have no effect on the N degradation parameters compared to the control treatment of no tannin. It is therefore likely that hydrolysable tannins do not provide protein protection in the rumen. This was further supported by the results of Hagerman *et*

al. (1992), who found that hydrolysable tannins are easily degraded in contrast to condensed tannin.

Makkar *et al.* (1997) examined the *in vitro* rumen degradability of N in white and coloured flowered varieties of faba beans (*Vicia faba*), and concluded that the white flowered varieties (low tannin) had a significantly higher degradability than their coloured flowered (high tannin) counterparts. In another *in vitro* study, Broderick and Albrecht (1997) assayed tannin free and tannin containing varieties of forage legumes for N degradation, and concluded that part of the variation in N degradability, between tannin containing and tannin free varieties, was associated with the presence of tannin but that other mechanisms affecting N degradation that were unassociated with tannins existed, particularly in red clover.

The effective degradabilities of N of the pea silages calculated at a rumen outflow rate of 0.05 h^{-1} were comparable to the mean result of 0.876 reported in the literature (Skiba *et al.*, 1996; Mustafa *et al.*, 2000; Mustafa *et al.*, 2002). Only the study by Skiba *et al.* (1996) calculated a corrected effective degradability of 0.702, which is comparable to the high tannin pea silage used in the current study, but lower than that of the low tannin pea silage.

4.4.3.2 Dietary parameters

The estimated dietary parameters determined using the UK metabolisable protein system (AFRC, 1993) indicated that the high tannin pea silage contained more rumen bypass protein compared to the low tannin pea silage, as indicated by DUP, and is indicative of the presence of condensed tannin (Makkar *et al.*, 1997). Perez-Maldonado and Norton (1996) reported that higher proportions of total dietary N reached the abomasa of sheep and goats in forage mixes containing condensed tannin compared to those not containing condensed tannin. The dietary parameters calculated here are similar to those reported by AFRC

(1993) for lucerne silage, of 135 g ERDP kg^{-1} DM and 20 g DUP kg^{-1} DM and that of 140 g ERDP kg^{-1} DM calculated from Dewhurst *et al.* (2003a) for white clover silage.

Table 4.14 compares the protein supply of whole-crop pea silage and some protein concentrates used in ruminant nutrition.

Table 4.14 Protein supply of whole-crop pea silage and protein concentrate feedstuffs determined at a rumen outflow rate of 0.08 h^{-1} (all g kg^{-1} DM)

Feedstuff	CP	ERDP	DUP	Reference
Whole-crop pea silage (mean)	**190**	**138**	**14**	**Current study**
Pea seed meal	252	172	42	AFRC (1993)
Linseed meal	379	242	87	
Rapeseed meal	400	265	78	
Soya-bean meal	497	260	193	

The ERDP level supplied by the whole-crop pea silage only represents 0.802, 0.570, 0.521 and 0.531g g^{-1} of that supplied by pea seed, linseed, rapeseed and soya-bean meals respectively. The DUP content supplied by whole-crop pea silage is significantly lower than that of pea seed, linseed, rapeseed and soya-bean meals. The DUP content supplied by whole-crop pea silage only represents 0.179 and 0.073 g g^{-1} of that supplied by rapeseed and soya-bean meals respectively. In order for whole-crop pea silage to supply the same amount of DUP as the additional 200 g FM soya-bean meal fed to the lambs in experiments 2b and 2c, the lambs would have had to consume, on average, 2.5 kg DM of whole-crop pea silage.

Estimates of dietary parameters for WCW of 60 g ERDP (0.08 h^{-1}) kg^{-1} DM was comparable to the 62 g ERDP (0.08 h^{-1}) kg^{-1} DM reported by AFRC (1993). However, the recorded DUP (0.08 h^{-1}) content of 8.8 g kg^{-1} DM was lower than that of 17 g kg^{-1} DM (AFRC, 1993). The value of 61 g ERDP (0.08 h^{-1}) kg^{-1} DM determined in the grass silage was surprisingly low when compared to a mean value of 95 g kg^{-1} DM (Table 1.14), and the high DUP (0.08 h^{-1}) level (18.7 g kg^{-1} DM) might have been a reflection of the late stage of maturity of the grass when it was harvested for ensilage.

4.4.4 Experiment 2b - Metabolism experiment

4.4.4.1 Intake, digestibility and energy balance

The mean forage DMI of 45.4 g kg^{-1} $W^{0.75}$ d^{-1} was lower than that of 58.6 g kg^{-1} $W^{0.75}$ d^{-1} recorded in lambs fed the same diets in the lamb growth experiment (Experiment 2c). The lower intakes recorded in the metabolism experiment, in comparison to the lamb growth experiment, are a reflection of the sheep consuming levels of feed close to maintenance, since they were housed in a temperature maintained building, under continuous lighting and their movement was restricted through use of metabolism crates. The mean apparent OM digestibility of the diets containing whole-crop pea silage was 0.765, which was considerably higher than that of 0.651 obtained by Fraser *et al.* (2001) feeding solely whole-crop pea silage. However, little difference was observed between apparent whole tract NDF digestibility, of diets containing whole-crop pea silage, with a value of 0.609 determined in this study and 0.585 determined by Fraser *et al.* (2001). Work by Adesogan *et al.* (2004), comparing pea-wheat bi-crops to grass silage reported that the bi-crops had a higher apparent OM digestibility than the grass alone, a result which concurs with that observed in the present study. The mean apparent whole tract GE digestibility of 0.749 determined for the diets containing whole-crop pea silage was significantly higher than that of 0.563 reported by Efe Serrano (1989), and higher than of 0.642 determined by Adesogan *et al.* (1994) in whole-crop wheat silages.

The measurement of ME through animals in metabolism experiments is more accurate than using predictive equations (McDonald *et al.*, 1998). The mean back calculated (from DE values) pea silage ME of 10.2 MJ kg^{-1} DM was higher than the 7.7 MJ kg^{-1} DM reported by Efe Serrano (1989), using sheep in metabolism crates, fed ensiled vining pea residue (containing a low grain proportion of approximately 0.1). The back calculated ME of the low tannin peas silage was numerically greater than that of the high tannin pea silage. This is in agreement with an *in vitro* study by Makkar *et al.*, (1997), who reported that low

tannin cultivars of *Vicia faba* had a significantly higher estimated ME, compared to the high tannin cultivars. The predictive equations used to determine ME, from laboratory measurements, in this study were derived for high temperature dried lucerne and not leguminous silages. The predicted ME of grass silage from laboratory measurements, using equation 4.2, was 1.53 MJ kg^{-1} DM higher than that measured through sheep (9.3 MJ kg^{-1} DM). However, the accuracy of this predictive equation is low, with a r^2 value of 0.20 (Givens *et al.*, 1989).

4.4.4.2 N balance and microbial protein synthesis

When N intake increased as a result of the inclusion of whole-crop pea silage or soya-bean meal, faecal N output remained unchanged, whereas urinary N increased. This result agrees with the results of Marini *et al.* (2004) who found that N excretion in the form of urine increased linearly with increased dietary protein, with no effect on faecal N, due to an increase in N recycling through the liver and increased kidney activity. The apparent digestibility of N was greater in the low tannin pea silage compared to the high tannin pea silage, possibly a reflection of the higher ADIN concentration in the high tannin pea silage. The inclusion of soya-bean meal to all forages increased apparent N digestibility, a reflection of its high nutritive value as a protein feedstuff.

The apparent whole-tract N digestibility of the pea silages of 0.766 was higher than that of 0.679 and 0.718 determined by Efe Serrano (1989) and Fraser *et al.* (2001), respectively. The apparent whole-tract digestibility of N was higher in the low tannin pea silage compared to the high tannin pea silage, an effect that might be attributable to increased tannin level. The proportion of dietary N retained by the lambs fed the pea silage mixtures in this study was 0.341. This is higher than the value of 0.079 determined by Efe Serrano (1989), and that of 0.192 determined by Fraser *et al.* (2001). Work with pea-wheat bi-crops by Adesogan *et al.* (2002), resulted in varying proportions of N retained from 0.174

to 0.312. Further work by Adesogan *et al.* (2004), using a short strawed variety of spring sown pea bi-cropped with spring sown wheat, resulted in a similar value of 0.369 to that found in the present study.

The levels of individual purine derivatives were unaffected by dietary treatment, a potential indication that the rumen micro flora were unaltered. The presence of an increased level of condensed tannin did not affect MPS, a finding in agreement with work summarised by Min *et al.* (2003). Verbic (2002) concluded that variability in MPS between forages was, in part, due to the difference in rumen pH. The levels of MPS, calculated on a g kg^{-1} digestible organic matter intake (DOMI), were unaffected by dietary treatment, with a mean value of 17.4 g kg^{-1} DOMI. This figure was comparable to the mean of 20.1 g kg^{-1} DOMI obtained in a study by Adesogan *et al.* (2004), with pea-wheat bi-crops determined in cattle.

4.4.5 Experiment 2c - Lamb growth

4.4.5.1 Intakes and performance

The mean grass silage intake of 58.3 g DM kg^{-1} $W^{0.75}$ d^{-1} of the lambs on the GS diets were higher than those of 26.2 g DM kg^{-1} $W^{0.75}$ d^{-1} and 43.4 g DM kg^{-1} $W^{0.75}$ d^{-1} reported by Fitzgerald (1996) and Speijers *et al.* (2005) respectively, for lambs fed good quality, medium precision chopped perennial ryegrass silage. The large difference between the results of this study and that of Fitzgerald (1996) may be explained by the difference in mean liveweight of the lambs. The lambs used by Fitzgerald (1996) had a heavier initial liveweight and slaughter weight of 37.5 and 48.9 kg respectively. Lambs in the current study consumed an average of 58.7 g DM kg^{-1} $W^{0.75}$ d^{-1} of the grass and pea silage mixtures, a figure comparable to those of Efe Serrano (1989) and Fraser *et al.* (2001), who reported silage intakes of 47.1 and 57.5 g DM kg^{-1} $W^{0.75\ d\text{-}1}$ respectively, for lambs fed *ad libitum* pea silage as their sole feed. Work by Castro *et al.* (2002) has shown that forage dry matter intake was unaffected by feeding concentrate or the frequency at which the concentrate was fed. There was no difference in FDMI between the two pea silages, a finding in contrast to Robbins *et al.* (1987b), who reported a reduction in dry matter intake with increasing tannin concentration. The higher FCE associated with diets containing soya-bean meal or whole-crop pea silage may also be associated with the increased ME supplied by these feedstuffs. There was a positive linear relationship observed between ADLWG and CP intake (CPI), determined by linear regression, where ADLWG $_{(g\ d^{-1})}$ = (0.8652 x CPI $_{(g\ d^{-1})}$) – 0.905 (r^2=0.986).

Feeding an additional 200 g d^{-1} of hi-pro soya-bean meal, to the GS diet (GS+HP), resulted in an increased growth rate of 73 g d^{-1} compared to those fed GS+LP, which equated to an increased growth rate of 2.74 g g^{-1} soya d^{-1}. The difference in growth rates between the lambs fed the HT+LP or LT+LP diet and the GS+LP diet was calculated as 32.0 and 37.2 g d^{-1} respectively, which equated to a soya replacement value of 87.6 and 101.9 g d^{-1}

respectively. The lambs fed the HT+LP and LT+LP diets had growth rates of 0.80 and 0.83 fold of those fed the GS+HP diet. When lambs were fed diets containing HT and LT pea silage with the additional 200 g d^{-1} of soya-bean meal, growth rates increased by 77 and 80 g d^{-1} respectively, figures which compare to the 73 g d^{-1} difference observed in the control diets. The difference between growth rates of lambs on pea silages with and without the additional 200 g soya-bean d^{-1} can be explained partially by daily metabolisable protein (MP) supply. Table 4.15 shows the mean daily intakes of ERDP, DUP and FME and calculated MP supply.

Table 4.15 Mean daily ERDP, DUP, FME and metabolisable protein (MP) supply of experimental diets fed during Experiment 2c

Diet	ERDP 5 (g d^{-1})	DUP 5 (g d^{-1})	FME† (MJ d^{-1})	ERDP:FME	Limiting factor	MP (g d^{-1})
GS+LP	104.2	19.0	12.6	8.3	ERDP	85.4
GS+HP	160.8	45.4	14.9	10.8	FME	140.2
HT+LP	136.5	14.0	12.6	10.8	FME	94.3
HT+HP	198.3	45.2	15.4	12.9	FME	143.4
LT+LP	140.3	15.4	13.4	10.5	FME	100.7
LT+HP	196.9	41.8	15.7	12.5	FME	141.8

† Fermentable metabolisable energy calculated according to AFRC (1993), based on laboratory measurements

Lambs fed the HP+LP and LT+LP had an increased daily MP supply of 8.9 and 15.3 g MP d^{-1} respectively compared to GS+LP, which corresponded to an increased growth rates of 32 and 37 g d^{-1} respectively, whereas lambs fed HT+HP and LT+HP had an increased daily MP supply of 3.2 and 1.6 g MP d^{-1} respectively compared to GS+HP, which corresponded to increased growth rates of 36 and 44 g d^{-1} respectively. Due to the negligible difference between MP supply of diets fed with the HP concentrate but the increased growth rate observed with diets containing whole-crop pea silage, it is plausible to suggest that there is more FME supplied by the whole-crop pea silage than predicted using equations based on laboratory measurements. Using the equation of Mertens (1992) to calculate total dietary non fibre carbohydrates by difference (NFC), the HT+HP and LT+HP diets supply, on average, 60 and 88 g NFC d^{-1} more, respectively, than the 492 g

NFC d^{-1} supplied by the GS+HP diet. It is also possible that excess protein supplied by the diets containing whole-crop pea silage may have been utilised for gluconeogenesis within the rumen, resulting in an increased FME supply (Lobley, 1992) and therefore an increased MP supply to the animal.

The soya replacement values demonstrate that substituting half of the grass silage for whole-crop crop pea silage (on a DM basis), can replace an average of 94.8 g soya d^{-1}, in terms of growth rate, it is therefore plausible to suggest that if all the grass silage had been substituted for whole-crop pea silage the proportion of soya-bean meal potentially replaced could range from 0.876 to 1.000.

4.4.3.3 Blood metabolites

Plasma βHB concentrations measured in this study were within the normal range of <0.9 mmol l^{-1} described by Radostits (2003), for weeks 0, 4, 6 and 8 on all experimental diets. Timing of blood sampling has been shown to affect plasma βHB concentration in sheep, with a maximum value being measured 3.5 h post morning feed (Witt *et al.*, 1999; Richardson *et al.*, 2003). Work by Fraser *et al.* (2004) did not find any difference in plasma βHB in lambs fed lucerne (a similarly degradable protein source to pea silage), red clover or perennial ryegrass. Differences between treatments at week 8 (point of slaughter) are not directly comparable to those of earlier weeks due to the different method of blood sampling (exsanguination compared to jugular venepuncture) and the lambs having been starved overnight.

The effect of dietary concentrate treatment on PUN was more marked, with all the sheep fed the HP concentrate having PUN levels above the normal range durings weeks 2,4 and 6 (Figure 4.4). It was expected that the inclusion of pea silage into the ration would increase PUN, due to the higher levels of ERDP supplied by these feedstuffs (Table 4.6). This was indeed the case with diets offered with the LP concentrate, but not those fed the HP

concentrate. This difference may be explained by the time at which the lambs were blood sampled. Lambs were fed the concentrate at 08:00 h and it was rapidly consumed, in approximately 5 min. They were then offered silage at 08:30 after refusals had been recorded. Due to the greater amount of concentrate fed, it is plausible that the lambs fed the HP concentrate consumed less forage than those fed the LP concentrate in the 1.5 h preceding weighing and then blood sampling. Elevated PUN levels are highly correlated with rumen ammonia levels (Lewis, 1957), and with increased CP intake (Marini *et al.*, 2004). Diets containing whole-crop pea silage had a greater corrected effective degradability, than those only containing grass silage. Since there was no statistical difference between FDMI in lambs fed any of the experimental diets, lambs consuming diets containing whole-crop pea silage would have a greater rumen ammonia concentration compared to those consuming grass silage. The increased PUN concentration reported by Marini *et al.* (2004), in lambs fed high levels of dietary CP, was associated with an increase in N recycling to the gastro intestinal tract. There was no difference between mean PUN concentrations of lambs fed either the low tannin or high tannin diets.

4.4.3.4 Carcass characteristics

Lambs fed the diets containing soya-bean meal achieved a heavier slaughter weight compared to those fed the LP concentrate and its inclusion marginally increased the hind leg diameter, which was used in this study as an indication of protein deposition. Work by Craddock *et al.* (1974) found no effect of increased protein supply on the carcass characteristics of lambs slaughtered at 59 kg liveweight (LW). In contrast, Sinclair *et al.* (1991) reported an increased CCW when lambs, slaughtered at approximately 40 kg LW, were fed an increased amount of CP. The increased chop length observed in the lambs fed the LT forage mix did not result in an increased eye muscle area. Increased growth rate has been shown by Chestnutt (1992; 1994) to be analogous with increased body fat deposition.

This was also seen in this current experiment, with the lambs consuming forage mixtures containing whole-crop pea silage growing faster and having an increased level of subcutaneous fat. There was a positive relationship observed between ADLWG and fat depth (FD) as determined by linear regression, where ADLWG $_{(g\ d^{-1})}$ = (0.0111 x FD $_{(mm)}$) + 0.2442 (r^2=0.397). Lewis *et al.* (2004) reported an increase in total subcutaneous fat weight when lambs were fed a high level of CP, an effect seen in this study when comparing forage mixtures but not concentrate type. The subcutaneous fat depths reported in this study were less than the mean of 10.8 mm reported by Chestnutt (1994), but comparable to the 2.82 mm reported by Witt *et al.* (1999) and the 2.5 mm reported by Speijers *et al.* (2005). The results obtained in this study suggest that there was little difference in carcass composition between those fed the GS+HP and those fed either the HT+LP or LT+LP diets, therefore showing that there were no detrimental effects of feeding whole-crop pea silage.

4.5 Conclusions

The increased level of condensed tannins recorded in the high tannin whole-crop pea silage resulted in a decreased immediately degradable fraction of N (0.744 cf. 0.785), an increased DUP content (15.0 cf. 7.4 g kg^{-1} DM), and similar ERDP (143 cf. 140 g kg^{-1} DM) when compared to the low tannin whole-crop pea silage, thus indicating a protein preservation effect attributable to the presence of condensed tannin. Both whole-crop pea silages had a rapid N degradability, and hence would benefit from being fed alongside a rapidly degradable energy source, such as a sugar source, to maximise ammonia capture by microbes in the rumen. On average, the whole-crop pea silages supplied 2.2 fold more ERDP and 0.68 fold less DUP than the grass silage used in this study.

The higher level of condensed tannin did not affect voluntary feed intake in either the lamb growth or the metabolism experiment, potentially indicating that there was no reduction in palatability as a result of increased condensed tannin in the whole-crop pea silage. There was no effect of tannins on the level of MPS.

Feeding whole-crop pea silage in the diets of growing lambs, in a 1:1 mixture with grass silage (DM basis), could replace 94.8 g d^{-1} of soya-bean meal. Lambs fed whole-crop pea silage had an increased growth rate of 37.4 g d^{-1} compared to those fed grass silage.

5.0 EXPERIMENT 3: UTILISATION OF WHOLE-CROP PEA SILAGE, DIFFERING IN TANNIN CONTENT, BY PREGNANT DAIRY COWS IN LATE LACTATION

5.1 Introduction

The results from Experiment 2 indicated that the inclusion of whole-crop pea silage in the diet of growing lambs improved growth rate and could replace 95 g d^{-1} soya-bean meal. Work by Salawu *et al.* (2002a) indicated that pea-wheat bi-crops can replace moderate quality grass silage in the diets of lactating dairy cows, whilst recent work by Adesogan *et al.* (2004) has shown that pea-wheat bi-crops can reduce the amount of concentrate fed from 8 to 4 kg d^{-1} in the diets of lactating dairy cows without affecting milk or milk constituent yield. The global market governs the cost and availability of soya-bean meal, as it is used in both human and animal nutrition (Merry *et al.*, 2001). Wilkins and Jones (2000) suggested that ground peas would not be a suitable supplement to grass silage, due to their rapid rumen degradation. However, they would suit being fed with a forage high in rapidly degradable energy. Additionally, it has been suggested by Broderick (1995) that the inclusion of forage legumes containing low levels (<50 g kg^{-1} DM) of condensed tannin can improve protein utilization in the rumen, thus reducing environmental impact.

The objective of this experiment was to evaluate the inclusion of whole-crop pea silage, varying in condensed tannin content, as an alternative protein source to soya-bean meal in the diets of late lactation, pregnant dairy cows.

5.2 Material and Methods

5.2.1 Silage production

The high and low tannin pea silages and fermented whole-crop wheat (WCW) silage were the same as those described in Sections 4.2.1.1 and 4.2.1.3 respectively. All three clamps were opened on 14th July 2003.

The grass silage (GS) was cut from a predominantly perennial ryegrass sward on 31st May 2003 and allowed to wilt for 24 h, chopped using a trailed forage harvester (John Deere 3625, Nottingham, UK), and received a microbial-based additive (a 50:50 mix of Axphast and Axcool, Biotal, Cardiff, UK) at the rate of 4 l t^{-1} fresh weight. The grass was ensiled in a concrete walled, roofed, bunker silo and was consolidated well and covered with a double layer of silage sheet and weighed down with car tyres. The clamp was opened on 7th July 2003.

5.2.2 Animals and experimental design

Eighteen mid-late lactation (mean 194 days in milk, s.d.=41), multi-parous (mean 3rd lactation, range 2nd to 6th) pregnant Holstein-Friesian dairy cows that were yielding, on average, 29.7 kg day^{-1} (s.d.=2.1, 7 day mean) were selected from the Harper Adams University College commercial herd. The cows were randomly allocated to one of two experiments (experiment 3a or experiment 3b; 9 cows per experiment). Within each experiment, cows were randomly allocated to one of three dietary treatments in a latin square design (Appendix 2). Each 28 d treatment period consisted of a 21 d adaptation period followed by a 7 d sampling period. The cows were housed indoors for the duration of the experiment with free access to fresh clean drinking water. The housed area was split into two passageways, a feed passage and a cubicle passage. Both passageways were fitted with automatic slurry scrapers activated six times a day. Cows had access to 22 cubicles,

which were limed twice weekly (Monday and Friday) and covered with screened sawdust thrice weekly (Monday, Wednesday and Friday). The experiment commenced on 8th July 2003 and ran for 13 weeks.

5.2.3 Experimental diet formulation

The experimental concentrate (HP, Table 6.1) was formulated to provide sufficient ME and MP for a 600 kg cow yielding 27 kg milk day^{-1} in week 18 of gestation, with a predicted silage DM intake of 12 kg day^{-1}, according to AFRC (1993). The silage component of the control diet was a 50:50 mix, on a DM basis, of first cut GS and WCW. The test diets maintained the ratio between GS and WCW (1:1) and were a 25:25:50 mix (DM basis) of GS, WCW and either high tannin (HT) or low tannin (LT) pea silage respectively. Cows in experiment 3a received the HT pea silage and those in experiment 3b received the LT pea silage. In order to test the ability of whole-crop pea silages to replace some of the protein provided by soya-bean meal, the soya portion of the concentrate (138 kg FM t^{-1}) was replaced with an equivalent weight of wheat (LP, Table 6.1). Both concentrates were formed into 3 mm diameter pellets (BOCM Pauls Ltd., Ipswich, UK).

The experimental diets offered to the cows in experiment 3a were:

Control diet *ad libitum* GS:WCW (50:50 DM basis) + 8 kg day^{-1} HP	GWH
Test diet 1 *ad libitum* GS:WCW:HT (25:25:50 DM basis) + 8 kg day^{-1} HP	HTH
Test diet 2 *ad libitum* GS:WCW:HT (25:25:50 DM basis) + 8 kg day^{-1} LP	HTL

The experimental diets offered to the cows in experiment 3b were:

Control diet *ad libitum* GS:WCW (50:50 DM basis) + 8 kg day^{-1} HP	GWH
Test diet 3 *ad libitum* GS:WCW:LT (25:25:50 DM basis) + 8 kg day^{-1} HP	LTH
Test diet 4 *ad libitum* GS:WCW:LT (25:25:50 DM basis) + 8 kg day^{-1} LP	LTL

Table 5.1 Raw material composition of the high protein (HP) and low protein (LP) experimental concentrates (kg FM t^{-1})

	HP	LP
Wheat	320.5	458.5
Maize	160.0	160.0
Sugar-beet pulp	100.0	100.0
Rapeseed meal	56.0	56.0
Sunflower extract	92.0	92.0
High protein soya-bean meal	138.0	0.0
Urea	14.5	14.5
Molasses	50.0	50.0
Protected fat	30.0	30.0
Oil	16.0	16.0
Limestone granules	5.0	5.0
Calcined magnesite	5.0	5.0
Di-calcium phosphate	7.0	7.0
Salt	5.0	5.0
Vitamins and minerals	1.0	1.0

5.2.4 Experimental procedure

5.2.4.1 Feeding

Prior to the first experimental period, cows had been at grass for at least 16 h a day and were offered 30 kg cow^{-1} d^{-1} of buffer feed indoors. The buffer feed was fed as a total mixed ration and its composition was (all g kg^{-1} FM), 650 maize silage, 100 dairy concentrate, 65 haylage, 50 sugar-beet pulp, 130 caustic wheat and 5 dairy minerals. The cows were housed a week prior to the commencement of the first experimental period. During the first week of housing, the cows were fed a diet of 50:50 FM basis of GS and maize silage (DM 304 g kg^{-1}, ME 10.7 MJ kg^{-1} DM, CP 72 g kg^{-1} DM by NIRS) through 18 roughage intake control (RIC, Insentec, Markenese, Holland) bins. Dairy concentrate (Milk Pro 18; BOCM Pauls Ltd., Ipswich, UK) was initially fed in the forage mix but as the week progressed a greater proportion of the concentrate was fed through out of parlour feeders (OPF; Insentec, Markenese, Holland) as described in Table 5.2.

Table 5.2 Amount of concentrate (kg cow^{-1} d^{-1}) fed in the forage mix and through out of parlour feeders (OPF) during the first week of housing

	Tue.	Wed.	Thur.	Fri.	Sat.	Sun.	Mon.
Forage mix	5	5	3	0	0	0	0
OPF	0	3	5	8	8	8	8

The concentrate was fed in two meals a day (minimum inter meal interval of 8 h) when cows received either 3 or 5 kg d^{-1} and three meals a day when they were fed 8 kg d^{-1} (minimum inter meal interval of 6 h) through the OPF.

The cows were fitted with collar-mounted transponders that were programmed at the beginning of each period to allow cows access to their specified concentrate and forage mix (Appendix 2). The forages were mixed at 10:00 h daily, and dispensed into 6 RIC bins per treatment using a mixer wagon (Keenan Compact 70, Richard Keenan, Ireland). Refusals were collected thrice weekly (Monday, Wednesday and Friday) and the daily feed allowance adjusted to 1.05x of calculated intake. To maintain the ratio of the forage components, a sub-sample of the forages was taken for DM determination twice weekly (Monday and Thursday). Sub-samples of the forages and concentrates were taken daily during the sampling week and stored frozen (-20°C) prior to subsequent analysis.

The RIC bins were calibrated using standard weights at the start of the trial and on the Friday preceding the sampling week and the OPF were calibrated twice weekly (Tuesday and Thursday). Forage and concentrate intake data was downloaded from the control computer daily (Sunday-Saturday) during the sampling.

5.2.4.2 Milking and milk sampling

Cows were milked twice daily at approximately 06:30 and 18:30 h through a 24 x 24 direct to line herringbone parlour, with automatic identification by pedometer transponders (Westfaliasurge Ltd., Milton Keynes, UK). Milk yield was recorded automatically, using calibrated flow meters, at each milking. After milking, cows were excluded from the cubicles for a period of approximately 30 min. During the sampling week, milk samples were taken from each cow, using in line milk samplers (Westfaliasurge Ltd., Milton Keynes, UK), during the Monday evening, Tuesday morning, Thursday evening and

Friday morning milking. Milk samples were transferred to 35 ml plastic snap top vials (Scientific Laboratory Supplies Ltd., Nottingham, UK) and two lactabs (26.4 mg potassium dichromate and 6.1 mg mercuric chloride per tablet; Thompson and Capper, Runcorn, UK) were added to each sample. Milk samples were stored at 4°C for no longer than 10 d prior to subsequent analysis.

5.2.4.3 Weighing and condition scoring

Cows were weighed and condition scored every Wednesday at 14:00 h. Prior to weighing, the weigh crate (IAE, Leek, Staffordshire, UK) was checked for accuracy using standard weights. Condition scoring was determined to the nearest quarter score by the same person using the method of Lowman *et al.* (1976).

5.2.4.4 Blood sampling

All animals were blood sampled by jugular venepuncture at 12:00 h on the Thursday of the sampling week. From each animal, one 10 ml lithium heparin vacutainer and one 7 ml potassium oxalate vacutainer were taken. Blood tubes were centrifuged at 1700 xg for 15 min, and the plasma transferred into micro centrifuge tubes and stored at –80°C prior to subsequent analysis.

5.2.5 Analysis

5.2.5.1 Feeds

Forage and concentrate samples were thawed and bulked within each sampling week prior to further analysis. A sub-sample of all feedstuffs were analysed for DM, ash, CP, EE, NDF, NCDG, WSC and starch. In addition, the forages were analysed for pH, NH_3-N, VFAs and condensed tannin as described in Chapter 2. Estimated ME was calculated by

NCD(G) measurements using Equation 3.1 for the whole-crop pea silages, Equation 4.2 for the whole-crop wheat and grass silage and Equation 4.3 for the concentrate samples.

5.2.5.2 Milk analysis

Milk samples were warmed to 40°C, inverted twice and analysed for fat, protein and lactose by infrared spectroscopy, using the whole milk channel of a Dairylab 2 (FOSS, Warrington, UK) milk analyser. The Dairylab analyser was calibrated using a range of standards (Q&M Ltd, Bury St. Edmunds, UK) varying in fat (247-629 g kg^{-1}) and protein (305-366 g kg^{-1}).

5.2.5.3 Blood analysis

Plasma samples were defrosted slowly and those preserved with lithium heparin were analysed for albumin, total protein and urea, whereas those preserved with potassium oxalate were analysed for glucose, beta-hydroxy butyrate (βHB) and non-esterified fatty acids (NEFA) as described in Section 2.13.

5.2.5.4 Calculations and statistical analysis

Nitrogen efficiency for milk production was calculated using Equation 5.1.

$$\text{N efficiency}_{g\,N\,g^{-1}\,N} = \frac{(\text{milk protein yield (kg d}^{-1}) \div 6.38)}{(\text{crude protein intake (kg d}^{-1}) \div 6.25)} \qquad \text{Equation 5.1}$$

All recorded parameters were analysed using the latin square analysis of variance procedure of Genstat (version 5, VSN, Cambridge, UK). The block structure used was cow x period and the treatment structure was diet. The data from cow 6 (experiment 3b) was excluded from statistical analysis due to drying off prematurely, which was not associated with dietary treatment. The data from cow 11 (experiment 3b) was also excluded from the second period due to health problems not associated with dietary treatment.

5.3 Results

5.3.1 Feed analysis

The four forages varied in DM ranging from 244 to 402 g kg^{-1} FM, with the HT and LT forages having a similar DM content (Table 5.3). The OM content of the WCW was higher (P<0.050) than the mean of the other three forages (Table 5.3), but there was little difference between forages in pH, with a mean value of pH 4.0. The CP content was lowest in the WCW and highest in the HT forage, with intermediate values for the GS and LT forages respectively (Table 5.3). There was no significant difference between forages in terms of NH_3-N, with a mean value of 154 g kg^{-1} TN. The WSC content of the GS and WCW were, on average, 13.6 g kg^{-1} DM lower than either the HT or LT forages (Table 5.3). The starch content varied greatly between forages with the GS containing 1 g kg^{-1} DM compared to the WCW that contained 223 g kg^{-1} DM, whilst the LT forage contained more starch than the HT forage (P<0.050, Table 5.3). The NDF content of the HT and LT forages were similar with a mean value of 254 g kg^{-1} DM, with increased levels observed in the WCW and GS forages (Table 5.3). The highest EE content was observed in the GS and the lowest in the WCW, with intermediate values for the HT and WCW forages (Table 5.3). Estimated ME was lowest in the GS and highest in the LT forage (Table 5.3). Tannin content was not determined in either the GS or WCW. The HT forage had 1.98 fold more (P<0.001) tannin compared to the LT forage (Table 5.3). The acetate content of the GS and WCW forages was higher (P<0.050) than either the HT or LT forages (Table 5.3). Propionate contents varied from 0.00 to 0.79 g kg^{-1} DM, with no butyrate or valerate being detected (Table 5.3).

Table 5.3 Chemical composition and estimated ME of the grass silage (GS), fermented whole-crop wheat silage (WCW), high tannin pea silage (HT) and low tannin pea silage (LT) fed during experiments 3a and 3b (all g kg^{-1} DM unless otherwise stated)

	GS	WCW	HT	LT	s.e.d.	*P*
DM (g kg^{-1} FM)	244^{a}	402^{c}	338^{b}	365^{b}	15.8	<0.001
OM	991^{a}	996^{b}	989^{a}	991^{a}	3.4	0.011
pH	4.1	3.8	4.1	4.1	0.11	0.092
CP	121^{b}	82^{a}	189^{d}	177^{c}	5.0	<0.001
NH_3-N (g kg^{-1} TN)	144	173	159	138	18.8	0.304
WSC	13.7^{a}	15.6^{a}	27.6^{b}	28.8^{b}	2.07	<0.001
Starch	1^{a}	223^{d}	58^{b}	87^{c}	7.8	<0.001
NDF	537^{c}	338^{b}	256^{a}	252^{a}	19.0	<0.001
EE	23.1^{b}	11.7^{a}	21.1^{b}	13.4^{a}	1.62	<0.001
Est ME† (MJ kg^{-1} DM)	11.4^{a}	11.6^{b}	11.8^{b}	12.1^{c}	0.10	<0.001
Tannin (TAE‡)	n.d.	n.d.	93.4^{b}	47.2^{a}	1.47	<0.001
VFAs						
Acetate	34.9^{b}	30.1^{b}	22.8^{a}	24.1^{a}	2.44	0.004
Propionate	0.79^{c}	0.29^{ab}	0.00^{a}	0.44^{bc}	0.167	0.009
Butyrate	0	0	0	0	-	-
Valerate	0	0	0	0	-	-

† Calculated from laboratory analysis using Equations 3.1 (peas) and Equation 4.2 (grass and wheat). ‡ Tannic acid equivalents (g kg^{-1} DM). Means in the same row not sharing a common superscript letter differ (P<0.050)

The chemical composition and estimated ME of the experimental concentrates are presented in Table 5.4. The concentrates had a similar DM, OM, WSC, NDF, EE and estimated ME. The HP concentrate contained an additional 46 g CP kg^{-1} DM compared to the LP concentrate, whereas the starch content of the LP concentrate was 57 g kg^{-1} DM higher (P<0.050) than that of the HP concentrate.

Table 5.4 Chemical composition and estimated ME of the high protein (HP) and low protein (LP) concentrates fed during the experiment (all g kg^{-1} DM unless otherwise stated)

	HP	LP
DM (g kg^{-1} FM)	883	885
OM	991	991
CP	225	179
WSC	46.8	49.5
Starch	239	296
NDF	189	153
EE	42.5	43.0
Estimated ME† (MJ kg^{-1} DM)	14.2	14.1

† Calculated from laboratory analysis using Equation 4.3. Means in the same row not sharing a common superscript letter differ (P<0.050)

5.3.2 Experiment 3a – Utilisation of high tannin pea silage

5.3.2.1 Dry matter intake

Cows fed the HTH diet consumed more ($P<0.050$) forage DM d^{-1} than those fed GWH, but there was no difference between HTL and either GWH or HTH (Table 5.5). Concentrate DM intakes were similar across all diets, with a mean value of 7.03 kg DM d^{-1} (Table 5.5). Total daily DM intake followed the same pattern as forage DMI (Table 5.5). Daily intakes of CP were lowest in cows fed the GWH and highest in those fed the HTH with an intermediate value for HTL (Table 5.5).

Table 5.5 Mean daily intake of dry matter and protein of cows fed either grass silage (0.50), whole-crop wheat (0.50) *ad libitum* and 8 kg high protein concentrate (GWH), or grass silage (0.25), whole-crop wheat (0.25) and high tannin pea silage (0.50) *ad libitum* with either 8 kg high protein concentrate (HTH) or 8 kg low protein concentrate (HTL) in Experiment 3a (kg d^{-1})

	Diet			Significance	
	GWH	HTH	HTL	s.e.d.	*P*
Forage DMI	12.0[a]	13.4[b]	13.0[ab]	0.53	0.041
Concentrate DMI	7.04	6.98	7.07	0.043	0.097
Total DMI	19.0[a]	20.4[b]	20.1[ab]	0.59	0.048
Crude protein intake	2.82[a]	3.52[c]	3.15[b]	0.069	<0.001

Means not sharing a common superscript, in the same row differ significantly ($P<0.05$)

5.3.2.2 Performance characteristics

Table 5.6 presents the mean daily performance data. There was no significant difference between cows fed GWH, HTH or HTL in milk yield, fat content, protein content or lactose content, fat yield, protein yield, lactose yield or 40 g kg^{-1} fat corrected milk yield. Nitrogen efficiency for milk production was highest ($P<0.001$) in cows fed GWH, and lowest in those fed HTH. There was no difference between cows fed GWH, HTH or HTL in terms or ADLWG or condition score change, with mean values of 0.85 kg d^{-1} and 0.032 $week^{-1}$ respectively.

Table 5.6 Mean milk yield, milk composition, nitrogen efficiency for milk production, ADLWG and condition score change of cows fed either grass silage (0.50), whole-crop wheat (0.50) *ad libitum* and 8 kg high protein concentrate (GWH), or grass silage (0.25), whole-crop wheat (0.25) and high tannin pea silage (0.50) *ad libitum* with either 8 kg high protein concentrate (HTH) or 8 kg low protein concentrate (HTL) in Experiment 3a

	Diet			Significance	
	GWH	HTH	HTL	s.e.d.	*P*
Milk yield (kg d^{-1})	23.1	23.8	22.0	0.86	0.123
Fat content (g kg^{-1})	43.1	43.1	43.8	0.87	0.669
Protein content (g kg^{-1})	35.7	35.4	36.1	1.15	0.847
Lactose content (g kg^{-1})	45.5	45.8	45.6	0.37	0.806
Fat yield (kg d^{-1})	0.99	1.02	0.96	0.036	0.274
Protein yield (kg d^{-1})	0.82	0.84	0.79	0.029	0.222
Lactose yield (kg d^{-1})	1.05	1.09	1.01	0.044	0.209
Fat corrected milk yield† (kg d^{-1})	24.7	25.6	24.1	0.89	0.264
Nitrogen efficiency (g N g^{-1} N)	0.289^{c}	0.234^{a}	0.246^{b}	0.0074	<0.001
Average daily live weight gain (kg d^{-1})	0.41	0.86	1.30	0.426	0.150
Condition score change ($week^{-1}$)	0.057	0.002	0.038	0.0336	0.290

† Corrected to 40 g fat kg^{-1}. Means not sharing a common superscript, in the same row differ significantly ($P<0.050$)

5.3.2.3 Blood metabolites

There was no difference between cows fed any diet in blood albumin concentration, with a mean value of 34.3 g l^{-1} across treatments, whereas cows fed GWH had the highest blood total protein concentration and those fed HTL the lowest, with an intermediate value for those fed HTH (Table 5.7). Plasma urea nitrogen was higher ($P<0.050$) in the cows fed the HTH compared to those fed either GWH or HTL (Table 5.7). There was no difference between dietary treatments on plasma concentrations of βHB, glucose or NEFA, with mean values of 0.715, 3.22 and 0.286 mmol l^{-1} respectively.

Table 5.7 Mean plasma metabolite concentrations of cows fed either grass silage (0.50), whole-crop wheat (0.50) *ad libitum* and 8 kg high protein concentrate (GWH), or grass silage (0.25), whole-crop wheat (0.25) and high tannin pea silage (0.50) *ad libitum* with either 8 kg high protein concentrate (HTH) or 8 kg low protein concentrate (HTL) in Experiment 3a

	Diet			Significance	
	GWH	HTH	HTL	s.e.d.	*P*
Albumin (g l^{-1})	34.7	34.4	33.8	0.60	0.319
Total protein (g l^{-1})	78.6^{b}	76.7^{ab}	74.4^{a}	1.07	0.006
Urea nitrogen (mmol l^{-1})	5.43^{a}	6.70^{b}	5.84^{a}	0.315	0.004
Beta-hydroxy butyrate (mmol l^{-1})	0.689	0.747	0.710	0.0701	0.708
Glucose (mmol l^{-1})	3.19	3.17	3.29	0.062	0.159
Non-esterified fatty acids (mmol l^{-1})	0.280	0.291	0.286	0.0088	0.500

Means not sharing a common superscript, in the same row differ significantly ($P<0.050$)

5.3.3 Experiment 3b – Utilisation of low tannin pea silage

5.3.3.1 Dry matter intakes

Cows fed diets containing the LT forage consumed an extra (P<0.050) 1.95 kg forage DM intake d^{-1} compared to those fed GWH (Table 5.8). There was no difference between cows fed GWH, LTH or LTL in concentrate DM intake, with a mean value of 7.03 kg DM d^{-1}. Cows fed diets containing the LT forage consumed, on average, 1.9 kg DM more (P<0.050) total DMI than those fed the GWH diet (Table 5.8). Daily intakes of CP were lowest in cows fed GWH and highest in those fed LTH, with an intermediate value for LTL (Table 5.8).

Table 5.8 Mean daily intake of dry matter and protein of cows fed either grass silage (0.50), whole-crop wheat (0.50) *ad libitum* and 8 kg high protein concentrate (GWH), or grass silage (0.25), whole-crop wheat (0.25) and low tannin pea silage (0.50) *ad libitum* with either 8 kg high protein concentrate (LTH) or 8 kg low protein concentrate (LTL) in Experiment 3b (kg d^{-1})

	Diet			Significance	
	GWH	LTH	LTL	s.e.d.	P
Forage DMI	11.6[a]	13.9[b]	13.2[b]	0.35	<0.001
Concentrate DMI	7.05	6.96	7.07	0.051	0.126
Total DMI	18.6[a]	20.8[b]	20.2[b]	0.35	<0.001
Crude protein intake	2.78[a]	3.52[c]	3.11[b]	0.054	<0.001

Means not sharing a common superscript, in the same row differ significantly (P<0.05)

5.3.3.2 Performance characteristics

Table 5.9 shows the mean daily performance characteristics. There was no difference between cows fed GWH, LTH or LTL in milk yield, with a mean value of 24.1 kg d^{-1}. The fat content of the cows fed LTH was lower (P<0.050) than those fed LTL, with an intermediate value for GWH. Protein content was not significantly affected by dietary treatment, with a mean value of 34.8 g kg^{-1}. There was a trend (P=0.065) for cows fed LTL to have a higher lactose content than those fed either GWH or LTH. There was no significant effect of dietary treatment on fat yield, protein yield, lactose yield or fat corrected milk yield. Nitrogen efficiency for milk production was highest (P<0.001) in

cows fed GWH and lowest in those fed LTH, with an intermediate value for LTL. There was no difference in ADLWG or condition score change between cows fed GWH, LTH or LTL, with mean values of 0.91 kg d^{-1} and 0.036 $week^{-1}$ respectively.

Table 5.9 Mean milk yield, milk composition, nitrogen efficiency for milk production, ADLWG and condition score change of cows fed either grass silage (0.50), whole-crop wheat (0.50) *ad libitum* and 8 kg high protein concentrate (GWH), or grass silage (0.25), whole-crop wheat (0.25) and low tannin pea silage (0.50) *ad libitum* with either 8 kg high protein concentrate (LTH) or 8 kg low protein concentrate (LTL) in Experiment 3b

	Diet			Significance	
	GWH	LTH	LTL	s.e.d.	*P*
Milk yield (kg d^{-1})	24.2	25.4	23.8	0.43	0.299
Fat content (g kg^{-1})	43.6^{ab}	41.9^{a}	45.7^{b}	1.04	0.011
Protein content (g kg^{-1})	35.4	34.4	34.7	0.42	0.112
Lactose content (g kg^{-1})	45.1	45.1	45.8	0.32	0.065
Fat yield (kg d^{-1})	1.05	1.02	1.08	0.026	0.105
Protein yield (kg d^{-1})	0.85	0.84	0.82	0.015	0.223
Lactose yield (kg d^{-1})	1.09	1.11	1.09	0.025	0.792
Fat corrected milk yield† (kg d^{-1})	26.3	25.5	26.9	0.64	0.108
Nitrogen efficiency (g N g^{-1} N)	0.300^{c}	0.234^{a}	0.259^{b}	0.0079	<0.001
Average daily live weight gain (kg d^{-1})	0.69	1.31	0.73	0.408	0.276
Condition score change ($week^{-1}$)	0.018	0.055	0.035	0.0352	0.603

† Corrected to 40 g fat kg^{-1}. Means not sharing a common superscript, in the same row differ significantly (P<0.050)

5.3.3.3 Blood metabolites

There was no difference between blood albumin and total protein concentration in cows fed any of the dietary treatments, with mean values of 34.4 and 76.0 g l^{-1} respectively (Table 5.10). Plasma urea nitrogen was highest (P<0.050) in cows fed LTH with no difference being observed between cows fed GWH or LTL (Table 5.10). There was no difference between dietary treatments in plasma concentration of βHB, with a mean value of 0.761 mmol l^{-1}. Plasma glucose concentration was highest (P<0.050) in cows fed diets containing the LT forage (Table 5.10). The highest (P<0.050) NEFA concentration was recorded in cows fed LTH, with no significant difference observed between GWH and LTL (Table 5.10).

Table 5.10 Mean plasma metabolite concentrations of cows fed either grass silage (0.50), whole-crop wheat (0.50) *ad libitum* and 8 kg high protein concentrate (GWH), or grass silage (0.25), whole-crop wheat (0.25) and low tannin pea silage (0.50) *ad libitum* with either 8 kg high protein concentrate (LTH) or 8 kg low protein concentrate (LTL) in Experiment 3b

	Diet			Significance	
	GWH	LTH	LTL	s.e.d.	*P*
Albumin (g l^{-1})	35.1	34.3	33.8	0.58	0.112
Total protein (g l^{-1})	75.6	76.0	76.5	1.13	0.704
Urea nitrogen (mmol l^{-1})	5.46[a]	6.47[b]	5.85[a]	0.249	0.006
Beta-hydroxy butyrate (mmol l^{-1})	0.822	0.698	0.762	0.0658	0.214
Glucose (mmol l^{-1})	3.19[a]	3.37[b]	3.41[b]	0.062	0.008
Non-esterified fatty acids (mmol l^{-1})	0.292[a]	0.313[b]	0.289[a]	0.0083	0.028

Means not sharing a common superscript, in the same row differ significantly ($P<0.050$)

5.4 Discussion

5.4.1 Silage composition

The whole-crop pea silages had stored well since the end of experiment 2b, with little spoilage being observed. The amount of silage removed to expose an unspoilt face was approximately 20 cm. Bolsen *et al.* (1993) showed that covering herbage with plastic sheet and old car tyres reduces the amount of spoilage formed during the ensiling process, but does not inhibit it all together. Woolford (1990) concluded that silages of good quality are more susceptible to aerobic deterioration than those of poor quality, and that deterioration occurs on the exposed faces quickly. During Experiments 2b and 2c, the pea silages were fed out manually at the rate of approximately 30 kg FM d^{-1}, whereas during Experiments 3a and 3b they were fed out mechanically at approximately 300 kg FM d^{-1}. This may potentially have led to greater deterioration being observed during experiments 2b and 2c due to aerobic spoilage. The mean chemical composition of the pea silage fed during Experiments 3a and 3b was higher in DM, OM, NH_3-N and WSC compared to the same silage fed during Experiments 2b and 2c.The moderate NH_3-N content (154 g kg^{-1} TN) and the absence of butyrate and valerate in the pea silages is a reflection of little or no secondary fermentation (Woolford, 1990). This study shows that whole-crop pea silages remain stable in the clamp for over 364 d. The pea silage within the clamps remained cool to the touch during the feed out period, demonstrating that they were aerobically stable.

The WCW had remained stable for over 357 d prior to opening and had a chemical composition comparable to the long straw WCW produced by Sinclair *et al.* (2003), produced under similar conditions. The WCW had a sweet, alcoholic smell, an indication of fermentation by heterofermentative lactic acid bacteria (McDonald, 1973). There was very little spoilage observed (<5 cm) on the WCW clamp face, an indication of the

inhibition of spoilage organisms caused by the high DM content of the crop at harvest (Woolford and Pahlow, 1998).

The grass silage produced for this experiment was fed out after a relatively short fermentation period of 36 d. The clamp in which the grass silage was made was south facing, resulting in prolonged exposure to solar radiation, thus decreasing its stability at feedout. This would not normally have been a problem since grass silage is predominantly fed as a winter feedstuff, but during the experimental period (July – October 2003) daily temperature regularly exceeded 20°C. The high NDF content (537 g kg^{-1} DM) of the grass silage was again indicative of the late cutting date, although it had a moderate DM (244 g kg^{-1} FM).

5.4.2 Voluntary dry matter intakes

5.4.2.1 Effect of diet

Selection between the forage components of the mixes by cows was not observed at any time during the experiment, since the collected refusals closely resembled the composition of the forage mixes. Forage DMI was higher in the diets containing pea silage in comparison to the control diet. Work by Phipps *et al.* (1995) has shown that offering cows forage mixtures, of two forages, increases forage DMI in a comparison to grass silage alone. The control diet (GWH) was a mixture of two forages, whereas the test diets were a mixture of three forages, so it may be possible that the inclusion of a third forage may increase forage DMI in comparison to two. However, the mechanisms controlling this increase are unclear. Salawu *et al.* (2002b) and Adesogan *et al* (2004) reported that mid lactation cows fed pea-wheat intercrops had a higher forage DMI compared to grass silage. The increases in forage DMI observed in this current study may be attributable to the lower NDF content of the pea silage mixes compared to the control diet (346 and 438 g NDF kg^{-1}

DM respectively) or the higher rumen degradability of pea silages compared to grass silage and WCW (Experiment 2).

The forage DMI recorded in this study were comparable to those reported by Salawu *et al.* (2002a) and Adesogan *et al.* (2004) for cows consuming pea wheat bi-crops, and those of Dewhurst *et al.* (2003b), for cows consuming leguminous silages, during winter-feeding trials. The cows used in the current study were pregnant and in late lactation. It is therefore plausible to suggest that DMI may have been limited due to the size of the foetus. Housing of lactating cows and restricting the forage component of the diet to silage is not normal management practise in the UK during the summer due to the abundance of grazing.

5.4.2.2 Effect of tannin

The mean forage DMI of cows fed the HT pea silage mixes in experiment 3a was increased by 1.02 fold compared to the control diet, whereas those fed the LT pea silage mixes in experiment 3b had an increased forage DMI of 1.17 fold compared to the control diet. This difference of 0.15 fold between the cows consuming the HT and LT forages and their respective controls is similar to the 0.12 fold difference observed by Waghorn *et al.* (1994b) in sheep consuming fresh harvested lotus, containing 55 g CT kg^{-1} DM, with or without an intraruminal infusion of the tannin binding compound polyethylene glycol (PEG). It was suggested by Waghorn *et al.* (1994b) that the reduction in DMI of the sheep without the PEG infusion was due to decreased ruminal turnover rather than decreased palatability caused by the CT.

5.4.3 Production characteristics

When the cows were selected from the college herd they were averaging 29.7 kg milk d^{-1} (s.d.=2.1), but a consequence of housing was to drop the mean milk yield by 1.50 kg after 1 week. Throughout the duration of the 12 week experimental period, milk yield dropped

by an average of 0.58 kg week^{-1} (r^2=0.976), equivalent to a weekly proportional drop of 0.021 kg kg^{-1}. This rate of decline agrees with the findings of Thomas and Rooke (1983), who stated that milk yield decreased at the rate of 0.020-0.025 kg kg^{-1} per week post peak. There was no difference between the milk yield of the animals fed the ensiled pea mixtures and their respective control group. The observed increase in forage DMI did not lead to an increase in milk production, which was in contrast to the meta analysis finding of Hristov *et al.* (2004). Hristov *et al.* (2004) evaluated 246 published dairy production studies and determined, through modelling, that total DMI was the major variable influencing milk production. Work by Bertilsson *et al.* (2001) and Dewhurst *et al.* (2003b), with early lactation cows, has shown that if baled grass silage is completely replaced with a baled whole-crop leguminous silage, mean total daily DMI increases 1.07 fold and mean milk yield increases 1.11 fold. However, if only half of the grass is replaced with a baled whole-crop leguminous silage, mean total DMI increases 1.07 fold and mean milk yield increases 1.09 fold. In this current study, the inclusion of whole-crop pea silage increased mean daily DMI by 1.08 fold with no difference in milk yield, an effect comparable to the results of Dewhurst *et al.* (2003a), with mid lactation cows fed red clover silage having a 1.11 fold increase in total DMI but only a 1.01 fold increase in milk yield compared to the grass silage control.

The experimental concentrate (HP) was formulated to meet the needs of the cows consuming the control diet (GWH). When using an ERDP:FME ratio of 11:1, as described in AFRC (1993), the cows fed the diets containing the pea silages were deficient in fermentable metabolisable energy whereas those fed the GWH were deficient in ERDP (Table 5.11).

Table 5.11 Mean daily ERDP, DUP, FME and metabolisable protein (MP) supply of experimental diets fed during Experiments 3a and 3b

Exp	Diet	ERDP 8 (g d^{-1})	DUP 8 (g d^{-1})	FME† (MJ d^{-1})	ERDP:FME	Limiting factor	MP (g d^{-1})
3a	GWH	1864	609	184	10.1	ERDP	1797
	HTH	2420	670	199	12.2	FME	2065
	HTL	2234	501	195	11.5	FME	1868
3b	GWH	1836	600	180	10.2	ERDP	1770
	LTH	2465	599	207	11.9	FME	2051
	LTL	2248	427	200	11.2	FME	1830

† Fermentable metabolisable energy calculated according to AFRC (1993), from laboratory measurements

The daily requirement for 1730 g metabolisable protein (MP), for which the diets were initially formulated, was exceeded by all of the offered diets. There was little difference (<72 g d^{-1}) between the MP supply of the pea silage mixes fed with the LP diet and the control diet, therefore indicating that the inclusion of whole-crop pea silage in the diet could match the MP supply from the control diet which contained 1 kg DM d^{-1} soya. However, when the pea silage mixes were fed with the HP concentrate, the MP supply increased by an average of 275 g d^{-1} compared to the control diet. The lack of response in production characteristics, i.e. milk protein content or growth rate, observed when feeding the whole-crop pea silage with the HP concentrate could be attributable to not having sufficient numbers of animals or the variation in stage of pregnancy.

Broderick (2003) explored the dietary interactions between feed energy and feed protein in early lactation dairy cows, and found that milk yield was more closely associated with increased non-structural carbohydrate intake, i.e. starch, than dietary protein. Feeding the LP concentrate resulted in an increased starch intake of approximately 400 g d^{-1} compared to the HP concentrate. The increased milk fat content of the cows fed LTL compared to those fed LTH did not result in any difference in daily fat yield. Work by Salawu *et al.* (2002a) found that milk constituent concentration decreased when cows were fed pea-wheat bi-crops, but further work by Adesogan *et al.* (2004) with pea wheat bi-crops reported no difference between treatments in milk constituent concentrations. Milk energy

output, calculated using the method of Tyrell and Reid (1965), resulted in no difference between diets, with the milk energy output of cows averaging 74.7 and 78.2 MJ d^{-1} for experiment 3a and 3b respectively (P=0.187 and 0.539 respectively). Due to the lack of reliable predictive equations for ME of WCW and pea silage it is not possible to calculate the energy efficiency of milk production. There was great variation in average daily live-weight gain ranging from 0.41 to 1.31 kg d^{-1}. The observation that these differences were not significant may be a reflection of the stage of gestation, which was not accounted for in the blocking structure. The condition score change of the cows was also unaffected by dietary treatment, but there was a change during the experimental period from a mean condition score of 2.78 to 3.17.

5.4.4 Blood metabolites

Plasma albumin and total protein (TP) concentrations are indicators of long-term protein status (Topps and Thompson, 1984). The albumin concentrations were unaffected by any of the dietary treatments, but cows in experiment 3a fed HTL had a lower TP concentrations than those fed GWH. This difference was not observed in experiment 3b. This was unexpected, since the cows fed GWH consumed 330 g CP d^{-1} less than those fed HTL. Increased levels of globulin may be associated with chronic mastitis (Ward *et al.*, 1995), but none of the cows had clinical mastitis during the sampling periods, but there may have been cows with sub clinical mastitis. Plasma urea nitrogen (PUN) concentrations were all higher than the normal range of 3.5 – 5.0 mmol l^{-1} defined by Ward *et al.* (1995), but cows fed the pea silage forage mixes and the HP concentrate had significantly higher PUN concentrations, a reflection of the increased ERDP supply from soya compared to wheat (Table 5.12 and AFRC, 1993). The elevated PUN concentrations are likely to a reflection of an increased rumen ammonia concentration (Lapierre and Lobley, 2001), due to an excess of ERDP and a limitation of FME (Table 5.11).

The levels of βHB determined over both experiments were below the standard maximum value of 0.9 mmol l^{-1}, defined by Ward *et al.* (1995), indicating that the cows were not energy deficient and not having to mobilise excess body fat reserves (Topps and Thompson, 1984). Plasma glucose concentrations were unaffected in Experiment 3a, but in Experiment 3b cows fed the diets containing the low tannin pea silage had increased plasma glucose concentrations compared to the control treatment. This observation is in contrast to work by Makkar *et al.* (1995), who demonstrated that the presence of condensed tannin (*in vitro*) increased the molar proportion of propionate, a glucogenic precursor, formed in the rumen and, in turn, would have increased plasma glucose concentration. Work by Putnam and Varga (1998) has shown that blood glucose concentration increases with increased CP supply, with no effect on NEFA concentrations, in late gestation dairy cows. Results from this study are in contrast to Putnam and Varga (1998), with no increase in blood glucose with increased CP intake. Increased concentrations of blood plasma NEFA are normally associated with an increase in plasma βHB concentration (Bowden, 1971). Since plasma βHB concentrations were unaffected by dietary treatment, the elevated plasma NEFA concentration observed in cows fed LTH compared with the control diet (experiment 3b) may potentially be explained by either time of feeding of the concentrate portion or the excitability of the animals at the time of blood sampling (Bowden, 1971) or increased glucose supply, as plasma βHB concentration is inversely related to plasma glucose concentration (Ward *et al.*, 1995).

5.4.5 Nitrogen efficiency

Nitrogen efficiency for milk production was consistently highest in the cows fed GWH across both experiments, with a mean of 0.295 g g^{-1}. This may have been a reflection of diet formulation, since the diets were formulated for the control diet (GWH), or the greater observed intakes of pea forage mixes with no subsequent effect on milk N output. The average N efficiency for milk production of diets containing whole-crop pea silage was

0.241 g g^{-1}, which was higher than that of 0.207 g g^{-1} for cows consuming pea wheat bi-crops (supplemented with 4kg concentrate, Adesogan *et al.*, 2004), and that of 0.209, 0.205 and 0.181 g g^{-1} for cows consuming ensiled red clover, white clover and lucerne respectively (supplemented with 8kg concentrate, Bertilsson *et al.*, 2001), but lower than that of 0.286 g g^{-1} for cows consuming 50% whole-crop pea silage and 50% concentrate (Mustafa *et al.*, 2000).

Broderick (1995) concluded that forage legumes containing moderate levels of condensed tannin would result in improved N efficiency. Cows fed HTH had increased N efficiencies than those fed LTH when compared to their respective control groups, with proportions of 0.810 and 0.780 respectively. The N efficiencies for the cows fed the pea silage mixes with the LP were similar between Experiments 3a and 3b (0.851 and 0.863 as a proportion of their respective control). It is proposed that increasing the supply of FME to diets containing whole-crop pea silage would increase N efficiency.

5.4.6 Practical implications

This study has shown that inclusion of whole-crop pea silage into the diets of pregnant, late lactation dairy cows can replace a daily portion of 1 kg DM soya-bean meal, without affecting production traits. Evidence from this study indicates that there was an oversupply of protein in the diets containing whole-crop pea silage. Therefore, if the whole-crop pea silages had been fed as a 50:50 mix on a DM basis with WCW it may be theorised that milk production would have increased, as demonstrated by Adesogan *et al.* (2004) with pea-wheat bi-crops, due to an increase in starch and FME supply. Utilisation of whole-crop pea silage containing a moderate level of condensed tannin can improve efficiency of milk production in comparison to a whole-crop pea silage containing less condensed tannin.

5.5 Conclusions

Late lactation, pregnant, cows offered forage mixes containing whole-crop pea silage had increased daily forage DMI, but there was no effect on milk yield, or milk component yield, or condition score change. There was no difference in the performance of cows fed any of the test diets, i.e. HTH, HTL, LTH, or LTL, compared to their respective control (GWH), except for N efficiency. This indicates that 1.1 kg FM soya-bean meal can be replaced with 1.1 kg FM wheat and whole-crop pea silage, which are both feedstuffs that are possible to produce on farm and are traceable.

Feeding the HT variety of whole-crop pea silage reduced voluntary forage DMI by 0.12 fold, compared to feeding the LT variety, without affecting production characteristics, along with an increased N efficiency. Therefore, feeding whole-crop pea silage containing an increased level of condensed tannin resulted in an increased nutrient efficiency compared to whole-crop pea silage containing less condensed tannin.

6.0 GENERAL DISCUSSION

The aim of this series of experiments was to evaluate ensiled whole-crop legumes for ruminants. Initially, both high and low condensed tannin (CT) varieties of peas and beans were studied in order to determine the most appropriate ensiling conditions, based on both the ensiling profile and the nutritive quality of the resultant silages. Subsequent studies focused on the ability of ensiled whole-crop peas, differing in CT content, to replace soya-bean meal as a protein source in ruminant diets and their effects on animal performance and the efficiency of nitrogen (N) utilisation.

6.1 Agronomic advantages and ensiling characteristics

Spring sown peas and beans harvested for ensilage are an attractive break/catch crop in both standard and organic farming systems, due to their relatively short growing season of 12 weeks (Experiments 1 and 2) and 14 weeks (Experiment 1) respectively, and their ability to fix N in the soil for the following crop (Bergersen, 1973). The literature review has established that peas and beans can be grown in most areas of the UK, but care is needed with the choice of varieties used in Scotland. Establishment of peas and beans requires the same machinery used for the establishment of cereals, although a suitable mower, such as a front mounted drum mower without conditioner, would need to be used, in order to minimise pod and leaf loss.

Crop DM yields were unaffected by CT content, but a greater yield was observed in the field scale study (Experiment 2) compared to the ensiling study (Experiment 1). The mean DM yield of 9.45 t ha^{-1} is greater than that which would be expected from a 2 cut grass silage receiving 200 kg N ha^{-1} (~7.5 t DM ha^{-1}) but less that that of 10.45 t DM ha^{-1} from a 3 cut silage receiving 300 kg N ha^{-1} (Chadwick, 2004).

The results also provide evidence that whole-crop pea (Experiments 1 and 2) and bean (Experiment 1) silages can be produced from grain varieties and not just forage varieties (e.g. Fraser *et al.*, 2001; Salawu *et al.*, 2001a), which is in support of the findings of Adesogan *et al.* (2004). However, extensive lodging occurred in the stands of peas grown in Experiment 2, which was also observed by Faulkener (1985) and Fraser *et al.* (2001). Crop lodging resulted in a reduction in harvestable yield due to an increased stubble height. As a result soil contamination appeared minimal, with little difference in organic matter contents of peas grown in Experiments 1 or 2. Pea varieties bred for grain production tend to have superior grain yield from a smaller plant in comparison to the more erect and leafy forage varieties (NIAB, 2001).

Harvesting can either be achieved using a trailed forage harvester (Experiment 1) or a self-propelled forage harvester, which is generally used for the production of fermented/urea treated whole-crop cereals (Experiment 2), both of which are commonly available from agricultural contractors. One disadvantage of growing annual legumes for ensilage is the 5 year rotation gap required in order to reduce pathogen build up (NIAB, 2001), which does not seem to be the case in perennial forage legumes (Halling *et al.*, 2001).

The leguminous silages produced throughout this study had good fermentation characteristics, with a low pH (<4.2), low to moderate NH_3-N (70-160 g kg^{-1} TN) contents and a low butyric acid (<5 g kg^{-1} DM) concentration, which have led to them being typically classified as lactate type silages (McDonald and Edwards, 1976). However, the lactic acid content of the silages was not determined. The high acetic acid content (>20 g kg^{-1} DM) of the pea silages and the relatively long period of time taken for a reduction in pH to less than pH 4.5 (~48 h) are indicative of the initial stages of ensiling being dominated by the hetero-fermentative lactic acid bacteria (McDonald *et al.*, 1991). However, it appears that homo-fermentative lactic acid bacteria must have dominated the later stages of fermentation in order to achieve a final mean pH of pH 4.1 (Woolford and

Pahlow, 1998). A typical, well preserved, untreated grass silage has a rapid decline in pH, reaching pH 4.5 in less than 36 h (Davies *et al.*, 1998). This difference between grasses and legumes can be explained by the differences in buffering capacity, with legumes being more highly buffered than grasses (McDonald and Henderson, 1962).

6.2 Nutritional value

The chemical composition of the whole-crop pea silages produced throughout this series of experiments has shown that, although DM was variable (208-365 g kg^{-1} FM) the CP concentration was relatively consistent, with a mean value of 186 g CP kg^{-1} DM, a concentration that compares well to the mean of 187 g CP kg^{-1} DM for values published in the literature (Table 1.10). The CP content of the whole-crop bean silages was, on average, 138 g CP kg^{-1} DM. This value was considered disappointing when compared to the mean of 204 g CP kg^{-1} DM for other published studies (Table 1.9), a factor that contributed to the later studies being carried out with whole-crop peas. The CP contents of a good grass/clover silage can be in the region of 170 g CP kg^{-1} DM (AFRC, 1993). The CP content of grass silages produced in the current work and by Davies *et al.* (1998) averaged 119 g CP kg^{-1} DM, a figure that corresponds to 0.64 fold the CP supply from whole-crop pea silage. The high predicted ME (from laboratory measurements, using an equation formulated for dried lucerne) of 12.1 MJ kg^{-1} DM for the whole-crop pea silage is higher than that of most ensiled forages, except for good quality baled grass (AFRC, 1993). The higher back calculated ME content of whole-crop pea silage (Table 4.9) may be due to the mean starch content of 71 g kg^{-1} DM, a figure above the typical <2 g kg^{-1} DM observed in ensiled grass or perennial leguminous silages (Dewhurst *et al.*, 2003b).

The degradation characteristics and metabolisable protein degradation parameters of whole-crop pea silages have been discussed in detail in Experiment 2. However, due to the high effective degradability of whole-crop pea silages it was concluded that these silages

would benefit from being fed alongside an energy rich, protein deficient feedstuff such as fermented WCW or maize silage.

6.3 Animal performance and soya replacement

There were no detrimental health effects observed in any animals consuming diets containing whole-crop pea silage. The increased protein content and feed conversion efficiency observed in lambs consuming diets containing whole-crop pea silage (Experiment 2c), coupled with no difference in forage dry matter intake, resulted in increased lamb growth rates compared to the grass silage controls. Therefore, using whole-crop pea silage to finish early born lambs more rapidly could be beneficial in terms of receiving an increased market value for early spring lambs (Chadwick, 2004), making lamb production more profitable. Including whole-crop pea silage into the rations of late lactation dairy cows increased forage dry matter intake by approximately 1 kg DM d^{-1} when offered *ad libitum*. However, no increase in production was observed, an effect that is atypical with the inclusion of whole-crop legumes into dairy cow rations (Table 1.19). Dewhurst *et al.* (2005) reported that restricting forage dry matter intake of dairy cows offered maize/red clover silage to 5 kg d^{-1} less than *ad libitum* intake resulted in similar milk yields compared to cows offered the silage *ad libitum*. Therefore, if the intake of whole-crop pea silage, fed with the low protein concentrate, had been restricted to the same level as those cows offered the control diet (GWH) *ad libitum,* their performance may have been maintained and profitability increased.

Studies reported in the literature have evaluated the use of pea seed meal as a replacement for soya-bean meal in the diets of growing lambs (Lanza *et al.*, 1999) and high yielding dairy cattle (Syrjälä-qvist *et al.*, 1981). Lanza *et al.* (1999) reported no difference in lamb growth or carcass composition when pea seed meal supplied the same amount of CP as

soya-bean meal. Syrjälä-qvist *et al.* (1981) also reported that there was little difference in growth rate, milk yield or milk component yield when pea seed meal supplied the same amount of CP as that from soya-bean meal in the diets of dairy cows. Results from this study have shown that a proportion between 0.475 and 1.000 g g^{-1} of soya-bean meal can be replaced by the inclusion of whole-crop pea silage into finishing lamb and dairy cattle diets respectively. Soya replacement rates of this magnitude could significantly reduce the cost of animal production and, in turn, increase profit margins, depending upon world market price of soya-bean meal and other protein concentrates.

6.4 Nitrogen efficiency

Plasma urea N concentration was higher in both cattle and sheep offered diets containing whole-crop pea silage. This observation emphasises the over supply of ERDP in relation to FME (Leng and Nolan, 1984; Lapierre and Lobley, 2001). Farm N efficiencies were calculated for sheep and cattle using the one-compartment model of Kohn *et al.* (1997), and are presented in Tables 6.1 and 6.2 respectively using the following assumptions;

Areas of grass, whole-crop peas and, where appropriate, whole-crop wheat (WCW) were calculated according to the ratio of DM in the diets offered, assuming that grass silage and whole-crop wheat yielded 7.5 and 11.0 t DM ha^{-1} respectively (Chadwick, 2004) and the high tannin and low tannin peas yielded 9.70 and 9.19 t DM ha^{-1} respectively (Table 4.2).

Fertiliser N requirement was calculated assuming that grass silage and whole-crop wheat silage required 200 kg N ha^{-1} (Chadwick, 2004) and the amount of N fixed by legumes was calculated assuming that 1 ha of a leguminous crop could fix 75 kg atmospheric N ha^{-1} (Bergersen, 1973).

Livestock units (LU) were calculated according to the mean daily forage intakes of sheep (Table 4.11) and dairy cattle (Tables 5.6 and 5.9) over 56 d (finishing time of sheep) and

100 d (over winter housing period of dairy cattle) respectively, where 1 LU is equivalent to 1 dairy cow or 10 lambs (Chadwick, 2004). Concentrate N was calculated from the CP concentration of the concentrates fed during Experiments 2c, 3a and 3b (Tables 4.7 and 5.5) multiplied up to the total number of animals fed over the declared periods. Meat N content of lambs was calculated from cold carcass weights determined in Experiment 2c (Table 4.13) and using the assumptions that carcass DM content was 292 g kg^{-1} FM and the N content was 70.83 g N kg^{-1} DM as determined by Witt (1998), with lambs slaughtered at a similar live weight. Milk protein output was calculated assuming that the daily protein yield of each cow was the same as that determined in Experiments 3a and 3b (Tables 5.7 and 5.10) and that it did not change over the 100 d housing period. Milk N content was calculated by dividing total milk protein output by the conversion factor of 6.38 (ARC, 1980). Nitrogen efficiencies were calculated by dividing total N output by total N input. Increased farm N balance is reflective of more N being converted into a saleable product rather than being retained on the farm.

Due to the higher yields from whole-crop peas, each ha of forage was able to sustain more sheep than on the respective grass silage control (Table 6.1). Farm N efficiencies for sheep (Table 6.1) were consistently higher in diets that contained whole-crop pea silage, due to the difference between N fertiliser applied and atmospheric N fixed. The diets containing soya-bean meal (HP) resulted in a lower overall N efficiency, an effect potentially attributable to the unbalanced ERDP and FME supply (Table 4.15).

Table 6.1 Farm nitrogen (N) efficiency of wether lambs consuming experimental diets offered in Experiment 2c (all kg N ha^{-1} unless otherwise stated)

	GS		HT		LT	
	LP	HP	LP	HP	LP	HP
Grass area (ha)	1.00		0.56		0.58	
Pea area (ha)	0.00		0.44		0.42	
N fertiliser	200		113		116	
N fixed	0		33		31	
Livestock units†	15.4	15.4	18.1	17.1	16.6	16.6
Concentrate N	79	201	93	223	85	216
Total N in	279	401	239	369	232	363
Meat N / Total out	58	61	68	71	62	72
N efficiency (kg kg^{-1})	0.208	0.152	0.285	0.192	0.267	0.198

† 1 lamb = 0.1 livestock unit

When whole-crop pea silage was grown on farm, the number of LU/cows decreased in relation to the control diets (GWH, Table 6.2). This was due to the increased forage dry matter intake observed with the inclusion of leguminous silage (Tables 5.6 and 5.8).

Table 6.2 Farm nitrogen (N) efficiency of dairy cattle consuming experimental diets offered in Experiments 3a and 3b (all kg N ha^{-1} unless otherwise stated)

	Experiment 3a			Experiment 3b		
	GWH	HTL	HTH	GWH	LTL	LTH
Grass area (ha)	0.59	0.31	0.31	0.59	0.30	0.30
WCW area (ha)	0.41	0.21	0.21	0.41	0.21	0.21
Pea area (ha)	0.00	0.48	0.48	0.00	0.49	0.49
N fertiliser	200	104	104	200	101	101
N fixed	0	36	36	0	37	37
Livestock units†	7.7	7.3	7.1	8.0	7.0	6.6
Concentrate N	196	148	180	203	142	169
Total N in	396	288	320	403	280	307
Milk N / Total out	100	91	93	107	90	91
N efficiency (kg kg^{-1})	0.253	0.316	0.291	0.266	0.321	0.296

† 1 cow = 1.0 livestock unit

However, overall N efficiency was higher when whole-crop pea silage was included in rations. Due to the lower number of LU supported by diets containing whole-crop pea silage, with or without soya-bean meal (Table 6.2), overall N efficiency increased.

Feeding the whole-crop pea silage with the higher level of condensed tannin did not result in any significant increase in N efficiency on farm, in comparison to feeding the low tannin whole-crop pea silage, which is in contrast to the findings of Broderick (1995). The differences that were observed were small, <0.010 g g^{-1}, and could be attributable to biological variation. They would have only been considered significant if they were >0.050 g g^{-1}.

6.5 Silage costing and recommendations

The calculated production costs of whole-crop pea silage, with a 2 and 3 cut grass silage system, are presented in Table 6.3. The assumptions made were that agricultural contractors carry out all mechanical operations, the fertiliser applied to the grass was 20:10:10 N, phosphate and potash, and it was applied in two dressings for the 2 cut silage and three dressings for the 3 cut silage (Chadwick, 2004). It was assumed that the establishment costs of the grass swards would be split over a six-year life. The costs for the grass silage sprays and fertiliser were based on average costs during 2004 as reported by Chadwick (2004), whereas the costs associated with sprays for the whole-crop pea silage were the actual costs incurred during its production at Harper Adams University College. The forage harvester costs for the grass silage were based on contractor costs for a trailed precision chop forage harvester, and those for the whole-crop pea silage on the contractor costs for a self propelled forage harvester. Fresh weight yields for grass silage were based on 2004 averages (Chadwick, 2004) and the DM yield was based on cutting at 250 g kg^{-1} FM, with the yields of whole-crop pea silage being those determined in Experiment 2. The cost per tonne of CP was calculated using a CP concentration of 170 g kg^{-1} DM (AFRC, 1993) for the grass silage and 192 g kg^{-1} DM for the whole-crop pea silage.

Table 6.3 Proposed cost analysis for the production of a 2 or 3 cut grass silage fertilised with 200 and 300 kg N respectively, or a single cut whole-crop pea silage (all £ ha^{-1} unless stated otherwise)

	Silage		
	2 cut grass	3 cut grass	Whole-crop pea
Establishment costs			
Sub soiling	0.00	0.00	19.20
Ploughing	5.53[1]	5.53[1]	33.20
Power harrowing	4.57[1]	4.57[1]	27.40
Sowing	2.56[1]	2.56[1]	21.50
Seed cost	14.17[1]	14.17[1]	72.00
Fertiliser cost	9.17[1]	9.17[1]	0.00
Spray cost	10.00[1]	10.00[1]	35.00
Fertiliser application	1.08[1]	1.08[1]	0.00
Spray application	1.55[1]	1.55[1]	9.30
Total establishment costs	48.63	48.63	217.60
Land rental	200.00	200.00	200.00
Total per year	248.63	248.63	417.60
Variable costs			
Fertiliser N cost	78.00	117.00	0.00
Fertiliser P+K cost	22.00	33.00	0.00
Spay cost	12.00	12.00	10.45
Fertiliser application	13.00	19.50	0.00
Spray application	18.60	18.60	9.30
Total variable costs	143.60	188.10	19.75
Harvest costs			
Mowing	34.30	51.60	17.20
Forage harvester	74.60	111.90	66.00
Tractors and trailers (x3)	92.40	138.60	46.20
Buckrake	30.00	45.00	15.00
Total cost	231.30	347.10	144.40
Total annual cost	623.53	783.83	581.75
Yield			
Fresh weight (t)	31.0	43.0	45.1
Dry matter (t)	7.5	10.75	9.45
Crude protein yield (t)	1.28	1.83	1.81
Cost per tonne DM	83.14	72.91	61.56
Cost per tonne CP	487.13	428.32	321.41

[1] assumes 6 year life. Complied with figures from Chadwick (2004).

Establishment costs of whole-crop pea silage were predicted to be 1.7 fold greater than the establishment cost of grass silage when compared on a yearly basis, but increased fertiliser costs and harvesting costs for grass silage lead to a higher production costs of £21.58 t^{-1}

DM and £11.35 t^{-1} DM for 2 and 3 cut grass silage respectively when compared to the production costs of whole-crop pea silage.

Production of 1 t CP (DM basis) from whole-crop pea silage cost 0.66 and 0.75 fold the costs from 2 and 3 cut grass silage respectively. Assuming that rapeseed meal and soya-bean cost £95 t^{-1} FM and £160 t^{-1} FM respectively and have respective dry matter contents of 890 g kg^{-1} and 905 g kg^{-1} (AFRC, 1993) and protein contents of 400 g kg^{-1} DM and 480 g kg^{-1} DM (AFRC, 1993), the cost of 1 t CP (DM basis) is £266.85 t^{-1} and £368.32 t^{-1} respectively. The calculated production costs of 1 t CP (DM basis) from whole-crop pea silage were similar (1.08 fold) to that of soya-bean, but 1.52 fold the cost of rapeseed meal. Figure 6.1 presents sensitivity data for purchased rapeseed and soya-bean meal in relation to the cost of 1 t CP from whole-crop pea silage.

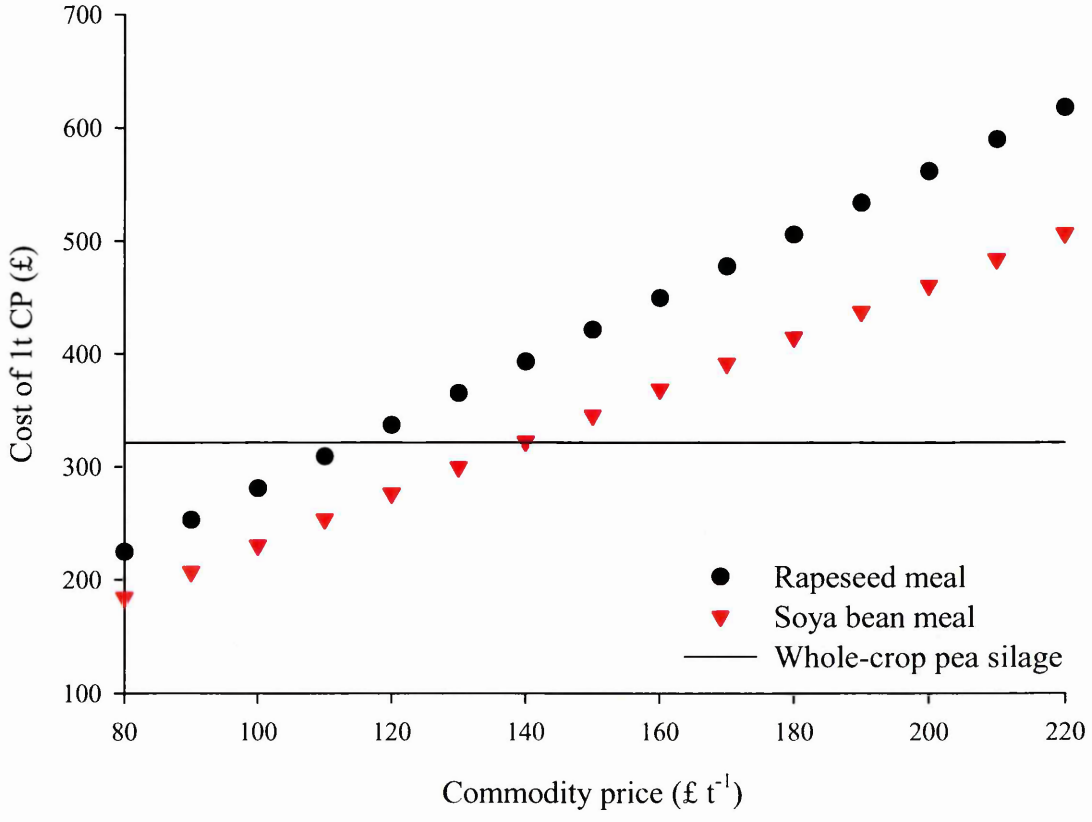


Figure 6.1 Relative costs of 1 t CP (DM basis) of rapeseed meal and soya-bean meal in relation to commodity price and whole-crop pea silage

In order for 1 t CP (DM basis) of purchased rapeseed meal and soya-bean meal to cost the same as 1 t CP (DM basis) from whole-crop pea silage, the cost of rapeseed meal would need to be less than £114 t^{-1} FM and soya-bean meal would need to be less than £139 t^{-1}. The cost of rapeseed meal is generally below this figure. However, soya-bean meal typically costs in excess of £140 t^{-1}. Inclusion of rapeseed meal and soya-bean meal are typically restricted to 15% and 25% concentrate intake of dairy cow rations (Ewing, 1997), whereas whole-crop leguminous silages can account for 100% of forage dry matter intake, which represents approximately 67% of total crude protein requirements (Bertilsson *et al.*, 2001; Dewhurst *et al.*, 2003b).

Using the proposed silage production costs in Table 6.5, and assuming that the production cost of fermented whole-crop wheat to be £77.22 t^{-1} DM, a profit margin has been calculated for the mean intakes of the diets offered in Experiments 3 and is presented in Table 6.4. The cost of the LP concentrate was assumed to be £140 t^{-1} (equivalent to a 16% CP as fed, standard dairy concentrate) and with an assumed wheat and soya cost of £80 t^{-1} and £160 t^{-1} respectively (McBurney, 2004) the HP concentrate cost £151 t^{-1}. Since there was no significant difference between any treatments in milk or milk component yield in Experiment 3, a baseline milk price of 19.0 pence per litre was assumed (FWI, 2005), with an average yield of 24 kg d^{-1}.

The cost of the forage was higher when whole-crop pea silage was fed. This was due to the increased forage dry matter intake observed in these diets. The total feed cost was lowest in the diet containing pea silage with the LP concentrate, representing a 7 pence per day per cow saving. Since milk yield was fixed for this comparison, the profit over feed cost of the pea silage forage mix with the LP concentrate was 0.3 pence per litre more than that of the control group (GWH).

Table 6.4 Calculated daily returns for a late lactation cow producing 24 kg milk d^{-1} fed either grass silage (0.50), whole-crop wheat (0.50) *ad libitum* and 8 kg high protein concentrate (GWH), or grass silage (0.25), whole-crop wheat (0.25) and mean pea silage *ad libitum*, disregarding tannin concentration, (0.50) with either 8 kg high protein concentrate (PH) or 8 kg low protein concentrate (PL)

	Diet			Contrasts†	
	GWH	PH	PL	PH	PL
Forage [1]	£0.86	£0.92	£0.88	1.07	1.03
Concentrate	£1.21	£1.21	£1.12	1.00	0.93
Total feed cost	£2.07	£2.13	£2.00	1.03	0.97
Milk sales[2]	£4.56	£4.56	£4.56		
Margin over feed	+£2.49	+£2.43	+£2.56	0.97	1.03
Margin over feed (pence per litre)	+10.4	+10.1	+10.7	0.97	1.03

† Relative proportion compared to GWH. [1] Based on mean performance data from Chapter 5. [2] At 19 pence per litre.

It must be remembered that the diets offered in Experiments 3a and 3b were formulated based on the metabolisable protein supply from the control diet (GWH). If the pea silage diets with the LP concentrate had been balanced for ERDP and FME, it is possible that the increased dry matter intake would have resulted in increased milk production. This could have been achieved by feeding the whole-crop pea silage with a higher proportion of fermented whole-crop wheat silage and reducing, if not excluding, grass silage from the diet. An increased performance may also have been observed if the cows had been fed the diet as a total mixed ration, 'balanced' for its supply and release of ERDP and FME (as described by Dewhurst *et al.*, 2000).

6.6 Future work

Further work is needed in order to evaluate a wider range of pea varities varying in condensed tannin content prior to assessing the effect of inclusion rate of whole-crop pea silage, and either whole-crop wheat silage or maize silage, in the diets of both growing lambs, beef cattle and lactating dairy cows. It would be advantageous to evaluate the production traits from early to mid lactation dairy cows consuming balanced diets containing whole-crop pea silage. Any further work with lambs would need to be based on

diets balanced for effective rumen degradable protein and fermentable metabolisable energy in accordance with AFRC (1993). To date, work with beef cattle fed whole-crop pea silage has been limited, but potential exists for whole-crop pea silage to be utilised in the diets of non-cereal fed beef in accordance with AFRC (1993).

Further work with dairy cows would need to be based on diets formulated according to Feed into Milk (FiM, Thomas, 2004), which does not balance diets on ERDP and FME, but instead balances diets on effective degradability of N and adenosine triphosphate (ATP) yield. In addition, FiM predicts a rumen stability value, which is linked to prevalence of rumen acidosis which is relevant for forages such as peas that have both a buffering capacity and starch content. Use of FiM calculates essential amino acid supply to the small intestine.

7.0 GENERAL CONCLUSIONS

- The production of low dry matter silage from whole-crop peas and beans is possible without an additive and without wilting. However, the optimum conditions determined for production of both whole-crop pea and whole-crop bean silages were following a 24 h wilt and without the use of an additive.

- Using high tannin/coloured flowered varieties of peas and beans reduced the extent of proteolysis that occurred during ensiling, in comparison to the low tannin/white flowered varieties.

- Whole-crop pea silage had similar *in sacco* degradation characteristics to lucerne silage, with a high proportion of rumen degradable protein. The higher tannin level reduced the immediately soluble protein fraction. However, no difference was observed in total rumen degradation.

- Diets containing whole-crop pea silage tended to have a higher farm N efficiency compared to control diets that were balanced for energy and protein supply.

- Feeding whole-crop pea silage did not adversely affect ruminant performance. In lambs, an improved feed conversion efficiency was observed. However, no improvement in dairy cow performance was observed, despite the increased forage dry matter intake of 1 kg d^{-1}.

- Feeding whole-crop pea silage in ruminant rations can replace between 44 and 100% of supplementary soya-bean meal. This can represent a major cost saving and has implications for both standard and organic farming enterprises.

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APPENDIX 1

In sacco incubation sequence used in Experiment 2a

date in	Time in	date out	Time out	Duration	S	H	E	P	A	S	H	E	P	B	S	H	E	P	C	S	H	E	P	D
24-Sep	08:00	24-Sep	12:00	4																				
24-Sep	12:00	24-Sep	20:00	8																				
24-Sep	20:00	25-Sep	12:00	16																				
25-Sep	12:00	25-Sep	20:00	8																				
25-Sep	20:00	26-Sep	20:00	24																				
26-Sep	20:00	27-Sep	12:00	16																				
27-Sep	12:00	28-Sep	12:00	24																				
28-Sep	12:00	30-Sep	12:00	48																				
30-Sep	12:00	02-Oct	12:00	48																				
02-Oct	12:00	05-Oct	12:00	72																				
05-Oct	12:00	08-Oct	12:00	72																				
08-Oct	12:00	08-Oct	14:00	2																				
08-Oct	14:00	08-Oct	16:00	2																				
08-Oct	16:00	08-Oct	20:00	4																				
08-Oct	20:00	09-Oct	08:00	36																				
09-Oct	08:00	10-Oct	20:00	36																				
		TOTAL		17.5																				

Low tannin pea silage
High tannin pea silage
Grass silage
WCW

APPENDIX 2

Latin square designs used in Experiment 3

Experiment 3a (high tannin pea silage)

	Period 1	Period 2	Period 3
Cow 1	HTL	GWH	HTH
Cow 2	GWH	HTH	HTL
Cow 3	GWH	HTL	HTH
Cow 4	HTH	HTL	GWH
Cow 5	HTL	HTH	GWH
Cow 10	HTH	GWH	HTL
Cow 12	HTL	GWH	HTH
Cow 13	GWH	HTH	HTL
Cow 17	HTH	HTL	GWH

Experiment 3b (low tannin pea silage)

	Period 1	Period 2	Period 3
Cow 6	LTH	GWH	LTL
Cow 7	LTL	LTH	GWH
Cow 8	LTL	LTH	GWH
Cow 9	GWH	LTL	LTH
Cow 11	LTH	GWH	LTL
Cow 14	LTL	GWH	LTH
Cow 15	GWH	LTL	LTH
Cow 16	LTH	LTL	GWH
Cow 18	GWH	LTH	LTL